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3. thyroid hormones  
4. parathyroid "  
5. gland "  
6. pancreas (7) oestrogens  
8. progestogens (9) androgens  
10. endocrinology  
11. endocrine disorders  
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# HORMONES IN CLINICAL PRACTICE





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BY

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# CONTENTS

CHAPTER	PAGE
LIST OF ILLUSTRATIONS . . . . .	vii
PREFACE . . . . .	ix
I ANTERIOR PITUITARY HORMONES . . . . .	i
II POSTERIOR PITUITARY HORMONES . . . . .	38
III THYROID HORMONE . . . . .	44
IV PARATHYROID HORMONE . . . . .	56
V THE SUPRARENAL GLAND HORMONES . . . . .	68
VI THE ISLET SYSTEM OF THE PANCREAS . . . . .	118
VII OESTROGENS . . . . .	133
VIII PROGESTOGENS . . . . .	169
IX ANDROGENS . . . . .	180
X THE PINEAL AND THYMUS . . . . .	204
XI ENVIRONMENTAL INFLUENCES ON GLAN- DULAR FUNCTION . . . . .	210
XII PSYCHO-SOMATIC ENDOCRINOLOGY . . . . .	212
XIII NON-HORMONAL SUBSTANCES FOR THE TREATMENT OF ENDOCRINE DISORDERS . . . . .	222
XIV VITAMIN-ENDOCRINE RELATIONSHIPS . . . . .	235
XV DIAGNOSTIC PROCEDURES . . . . .	248
XVI COMMERCIAL PREPARATIONS . . . . .	355
INDEX . . . . .	369





# LIST OF ILLUSTRATIONS

FIG.	PAGE
1. The Interrelationship of Glands and Hormones . . . . .	xii
2. The Anterior Pituitary Cycle . . . . .	I
3. The Menstrual Cycle . . . . .	16
4. The Anterior Pituitary-Ovarian Cycle in Relation to the Thyroid and Adrenal Cortex . . . . .	29
5. The Role of the Endocrine Glands in Blood Sugar regulation .	124
6. The Effect of increased Oestrogen or Thyroxin Production on the Anterior Pituitary Gland . . . . .	141
7. The Effect of Oestrogen or Thyroxin-deficiency on the Anterior Pituitary Gland . . . . .	141
8. Kearn's Pellet Injector . . . . .	149
9. Infiltration of Procaine . . . . .	150
10. Insertion of Cannula and Trocar . . . . .	150
11. Insertion of Pellets after withdrawal of Trocar . . . . .	151
12. The Effect of increased Progestogen, Androgen or Adrenal Cortical Hormone production on the function of the Anterior Pituitary . . . . .	170
13. The Effect of Progestogen and Adrenal Cortical deficiency on the function of the Anterior Pituitary . . . . .	170
14. Cerebro-glandular interaction . . . . .	212
15. The Effect of Thiouracil on the Thyroid and Anterior Pituitary Gland . . . . .	223
16. Peri-renal insufflation . . . . .	291
17. Vaginal smear. Complete cytolysis . . . . .	293
18. Vaginal smear. Mucoid-cornified type . . . . .	294
19. Vaginal smear. Follicular phase . . . . .	295
20. Vaginal smear. Late follicular phase . . . . .	296
21. Vaginal smear. Post-ovulatory phase . . . . .	296
22. Vaginal smear. Late luteal phase . . . . .	297
23. Vaginal smear. Early pregnancy . . . . .	299

FIG.	PAGE
24. Vaginal smear. Pregnancy 12 weeks . . . . .	300
25. Vaginal smear. Pregnancy 20 weeks . . . . .	300
26. Vaginal smear. Pregnancy 36 weeks . . . . .	301
27. Vaginal smear. Menopause . . . . .	302
28. Vaginal smear. Post-menopause . . . . .	302
29. Endocervical smear of 62-year-old patient . . . . .	305
30. Cervical biopsy. Anaplastic carcinoma . . . . .	305
31. Endocervical smear of 43-year-old patient . . . . .	306
32. Cervical biopsy. Rapidly growing infiltrating squamous cell carcinoma . . . . .	306
33. Endocervical smear of 38-year-old patient . . . . .	307
34. Cervical biopsy. Rapidly growing squamous cell carcinoma . . . . .	307
35. Endocervical smear of 58-year-old patient . . . . .	308
36. Diffuse adenocarcinoma of uterus . . . . .	308
37. Endocervical smear of 62-year-old patient . . . . .	309
38. Cervical biopsy. Pre-invasive carcinoma . . . . .	309
39. Endocervical smear of 42-year-old patient . . . . .	310
40. Cervical biopsy showing pre-invasive squamous cell carcinoma . . . . .	311
41. Endocervical smear showing radiation changes . . . . .	312
42. Endocervical smear showing radiation changes . . . . .	312
43. Variations in vaginal temperature . . . . .	317
44. Variations in vaginal and oral temperature . . . . .	317
45. Endometrium in follicular phase . . . . .	324
46. Endometrium in presecretory phase . . . . .	324
47. Endometrium in secretory phase . . . . .	325
48. Endometrium showing cystic glandular hyperplasia . . . . .	325
49. Normal measurements in relation to age . . . . .	346
50. Average weight for women based on height and age . . . . .	347
51. Osseous centres and epiphyseal closure . . . . .	348
52-7. Radiographs of osseous maturation . . . . .	349-354



## PREFACE

THE scope and objectives of this book call for a brief explanation. It is a commonplace that the field of endocrinology is continually being enriched with new data, clinical and experimental, some confirming previous results and hypotheses, others contradicting them. At frequent intervals, then, to meet the needs of the general physician, it becomes necessary to make a fresh survey of the material scattered through the vast and ever-growing literature and to discard or modify concepts that do not accord with the facts established to date. The process is one that demands not merely a synthesis of new data with old but a fresh evaluation of therapeutic procedures involving the use of hormones or directed to a correction of endocrine disturbances. This is the task I have attempted; and I acknowledge with gratitude how much my efforts owe to the good fortune that has enabled me to study, and to discuss at first hand, the views prevalent on both sides of the Atlantic. When the first draft of the book was completed I was working in London as research endocrinologist to the Department of Obstetrics and Gynaecology, British Post-Graduate Medical School; and the text was drastically revised and the proofs corrected in the course of my duties as Research Associate in the Department of Endocrinology, University of Georgia.

For those occupied in general practice and even for some beginning to specialize in endocrinology, the study of the subject, with its conflicting data, confusing terminology and chaos of commercial preparations, must always be perplexing and sometimes disheartening. The resolution of these difficulties is not made any easier by the fact that a specialty so intricate in its content and so rapid in its development cannot be presented in small compass without risk of adding to the confusion or, even worse perhaps, of creating a delusive oversimplification. The fact must be faced that the knowledge set out in the general run of textbooks yields insufficient information on the practical application of hormonal substances for the treatment of endocrine disorders. On the other hand, it is possible—and this is what is attempted here—to review the more recent material within the framework of accepted knowledge, without producing another textbook of endocrinology; and to do so in a manner designed to bring to the busy practitioner precise information on such problems as the conditions for which hormone therapy is necessary or desirable, the choice of preparations, and the dosages in which they should be employed. Matters bearing so closely on everyday practice can and should be set out very simply and in a form adapted to rapid refer-

ence. Other matters, however, cannot be discussed without making considerable demands on the reader's attention, and the reviews of apparently contradictory data, as well as the extensive bibliographies, are intended for those who seek a deeper knowledge of the subject.

Anyone confronting the task of reducing a bewildering mass of data to some intelligible order must inevitably construct a pattern to which the material can suitably be fitted. In this work the facts about every hormone are set out, with minor divergencies, in an invariable sequence, beginning with the histology of the gland and the physiology of its secretion, and passing by way of its relation to other hormones or factors, its sources, standardization and clinical preparations, to a full discussion of its applications in practice, methods of administration and dosage. All the preparations mentioned in the text are included in the full list, given in Chapter XVI, of commercial preparations available in Britain, America, or both; and, in an endeavour to dispel the confusion resulting from their sheer diversity, full information is given about their potencies and the units in which they are expressed, and about their effects and the duration of their activity by different routes of administration. A full critical survey is also made of the diagnostic methods used in endocrinological practice.

This volume is thus offered not as yet another textbook of endocrinology but rather as a work of reference embodying the most recent data on the physiology of the hormones and their use in clinical practice; and as such the author hopes it will prove a useful guide in a difficult and far from completely charted territory.

I am very sensible of the debt I owe to the many colleagues, in England and America, with whom I have discussed the work in all stages of production. In particular I should like to thank Mrs. M. Rainbird and Miss J. Walters of London for their close collaboration in the preparation of the manuscript, and Miss J. Sirmans of Augusta, Georgia, for her great help in the revision of the galleys. To Dr. Harrison of London and Dr. P. Wermer of Chicago, both of whom were kind enough to read and suggest corrections to the typescript, I am grateful for much valuable advice; and to Dr. S. A. Singal for his generosity in drawing the charts. I am especially indebted to Dr. Maurice Newfield without whose original encouragement and detailed criticism of the text the work would not have been completed.

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If I may end on a personal note, there is one debt that could never be adequately acknowledged. It is to my wife whose help, not merely in technical matters such as the preparation of the photomicrographs, but in the countless details that go to the making of a book, has been an unfailing stimulus from the inception of the work to the passing of the last proof for press.

H. E. NIEBURGS





## CHAPTER I

### ANTERIOR PITUITARY HORMONES

THE anterior lobe of the pituitary consists of two main groups of cells, the chromophobes (neutrophils) and chromophils. The chromophobes are smaller than the chromophils. They contain a non-granular, poorly-staining homogeneous cytoplasm, and a small round nucleus rich in chromatin. They constitute about half of all the cells in the adult human hypophysis and appear to have no secretory activity.

The chromophils consist of two types of cells named acidophils

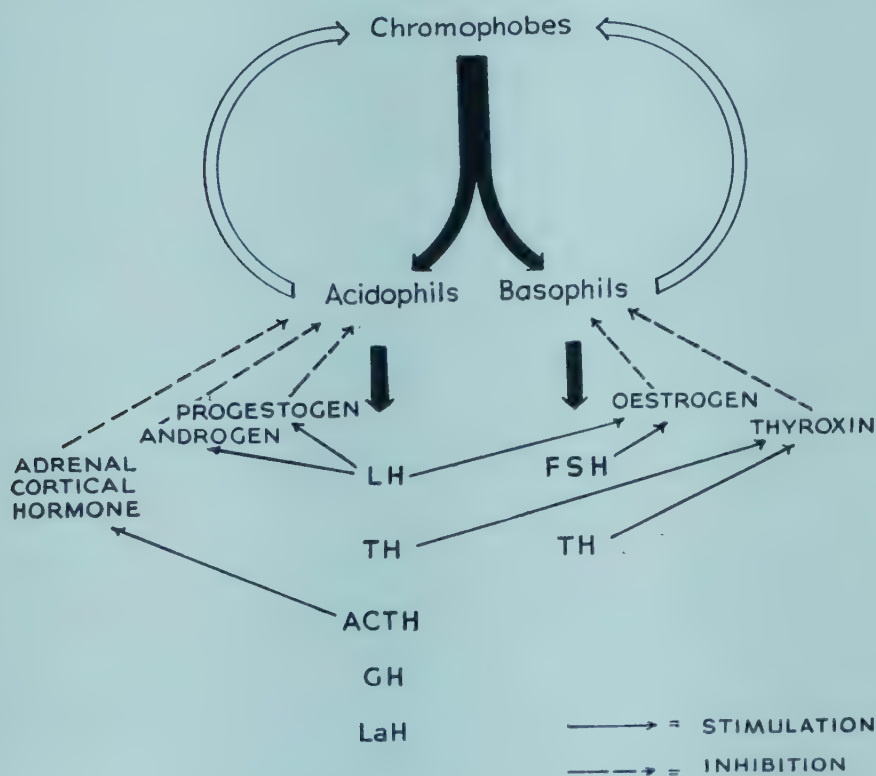


FIG. 2.—THE ANTERIOR PITUITARY CYCLE

(or eosinophils) and basophils. The acidophils represent about 43 per cent of the cells of the anterior pituitary in the non-pregnant woman. The basophils comprise 11 per cent of all the cells in the male and 7 per cent in the female. According to Severinghaus [1], the cells of the anterior pituitary show cycles of secretion. These cells elaborate their endocrine products, store them in gradually-increasing amounts in the cytoplasm, discharge them and then begin to elaborate and accumulate a new store of granules. On losing their granules, the

chromophils become chromophobes, while chromophobes, which accumulate specific granules, become acidophils or basophils as the case may be.

The basophils have been associated with the production of the follicle-stimulating (FSH) and thyrotrophic hormones, and the acidophils with the luteinizing (LH), growth, corticotrophic and lactogenic hormones (see Fig. 2).

Many hormonal actions have been ascribed to the pituitary gland, but there is no unanimity as to how many separate hormones the pituitary actually produces. A number of fractions, of a certain degree of purification, have been extracted from the pituitary gland and introduced for clinical use. The pituitary hormones, however, are proteins, a fact which presents great difficulty in their purification. In clinical use they may cause allergic reactions; and their prolonged use may be followed by antihormone formation. None of the pituitary hormones has yet been synthesized. The following hormones of the anterior pituitary have been obtained for clinical application:

1. Growth Hormone.
2. Thyrotrophic Hormone.
3. Corticotrophic Hormone.
4. Lactogenic Hormone.
5. Gonadotrophic Hormones.

## GROWTH HORMONE

### PHYSIOLOGY

Insufficient production of growth hormone causes retardation of growth, resulting in dwarfism, whereas increased activity of eosinophilic cells before epiphyseal union causes gigantism, associated with eunuchoidism in cases of delayed union of the epiphyses. Acromegaly results when, owing usually to adenoma of the eosinophilic cells, hyperfunction sets in after the epiphyses have united.

It has been observed that growth hormone decreases the non-protein nitrogen and amino-acid nitrogen of the blood and also the urinary total nitrogen [2]. Its growth-promoting property has been attributed to its action in increasing the amount of nitrogenous material available for cell growth and multiplication [3].

Knowledge of the physiology of growth hormone is based on experimental evidence, but this has been derived from the use of crude extracts contaminated by the adrenotrophic, thyrotrophic and lactogenic fractions.



Recently Li and Evans [159] have been able to isolate a protein from the anterior lobes of ox pituitaries which is apparently pure. In hypophysectomized treated rats an increase of 10 grams was produced by injections of 0.01 mg. daily, whereas untreated controls did not resume body growth. The endocrine glands were not increased in proportion to body weight, which indicates that the substance used was not contaminated with any other hormone [160]. The final evaluation, however, of this pure substance has to be postponed until extensive clinical data are available.

#### RELATIONSHIP OF THE GROWTH-PROMOTING FACTOR TO OTHER HORMONES

##### **Thyroid Gland Hormone**

A properly functioning thyroid gland is most important in the regulation of growth.

##### **Oestrogen**

High doses of oestrogen inhibit secretion of the growth hormone [4].

##### **Androgen**

High doses inhibit growth; small doses enhance growth considerably, owing to the effect of the androgen on nitrogen retention and on metabolism generally [5, 6] (see Chapter IX).

#### STANDARDIZATION

Growth-hormone extracts are standardized by their growth-promoting ability in hypophysectomized and intact rats. None of the units employed is comparable by definition alone, and no common standard is available.

#### PREPARATIONS FOR CLINICAL USE

The pituitary growth hormone on the whole has given very unsatisfactory results. Its frequent administration and possible antihormone formation make its application at this time undesirable. Although a pituitary growth preparation may in some cases produce slight growth, it will, when administered alone, never restore a dwarf to anything near to normal height, since growth is the effect of combined pituitary, thyroid, adrenal and gonadal activity. Shelton [8] reports only one instance in which, in his view, the patient grew as a result of the administration of anterior pituitary extract.

The preparations available are standardized in various units, some containing:

20 I.U. in 1 c.c.  
10 r.u. in 1 c.c.  
100 growth units (Collip) in 1 c.c.

#### INDICATIONS

Pituitary dwarfism provided that epiphyseal closure has not taken place.

#### DOSAGE

High doses of intramuscular injections as frequently as possible are indicated. The usual dose is 2-5 c.c. daily. The available evidence indicates that antihormone formation is very unlikely; but since the question is still open, rest periods are recommended.

In addition, concentrated vitamin preparations containing Vitamins A and D and thiamine should be given.

Thyroid in doses of  $\frac{1}{4}$  to 1 grain 2-3 times daily is a useful adjunct in the treatment of retarded growth.

Some clinicians advocate the use of androgens in the treatment of retarded growth, particularly when associated with hypogonadism (see Chapter IX). Small doses of testosterone propionate (5-10 mg. 3 times a week), or methyl testosterone (5 mg. sublingually daily), with fortnightly or monthly rest periods, are believed to exert a stimulating effect on the eosinophilic cells. Inhibition of the anterior pituitary gland with stunting of growth following androgenic treatment is apparently due to large protracted dosage.

In males with dwarfism due to partial pituitary deficiency a better growth-promoting effect is obtained if androgen administration is combined with gonadotrophin therapy [161-3]. Several courses of chorionic gonadotrophin are given in doses of about 500 I.U. 2 or 3 times per week for 8-10 doses with a 1-month rest interval between courses. This treatment induces retention of nitrogen, inorganic phosphorus, sulphate and calcium and promotes temporary retention of sodium chloride and water.

No convincing evidence is available that oestrogens stimulate growth in the female.

## TREATMENT OF DISORDERS DUE TO EXCESS OF GROWTH HORMONE

**Acromegaly**

Deep X-ray therapy or, when indicated, hypophyseal surgery is recommended. Some workers [147, 148] claim beneficial results from the administration of oestrogens and androgens. To inhibit increased pituitary function, large doses have to be employed over a prolonged period. Hutton and Reiss used oestradiol benzoate 1.5–10 mg. intramuscularly daily, followed by oestrone 1.0–1.5 mg. orally daily. Studies on the output of creatine and creatinine in acromegalic subjects has furnished convincing evidence of the inhibitory effect of large doses of oestrogens and androgens on the anterior pituitary lobes.

The excretion of creatine and creatinine in the urine is greatly increased in acromegaly during the active phase and is apparently due to anterior pituitary hyperfunction [150, 154–8]. Schrire and Scharpey-Schafer [149] produced a drop in the urinary excretion of creatinine following the administration of oestradiol benzoate 100,000 I.U. intramuscularly daily to two acromegalic women, and testosterone propionate intramuscularly 100 mg. daily to two acromegalic men. They regard these findings as evidence of pituitary inhibition by the sex hormones. There is ample proof that a rise in creatinine excretion is due to the increased release of some pituitary factor [150, 151, 152] and it is reasonable to assume that decreased creatinine excretion following the administration of sex hormones is caused by pituitary inhibition.

## THYROTROPHIC HORMONE

This hormone is extracted from the anterior pituitary lobes of animals in almost pure form.

## PHYSIOLOGY

Hypophysectomy, in experimental animals, has been found to lead to atrophy of the thyroid gland. The thyrotrophic hormone, when injected into certain animals, can reproduce all the signs and symptoms of toxic diffuse goitre, including exophthalmos. The administration of thyroid depresses thyrotrophic activity of the rat pituitary to less than 5 per cent of the normal level [164].

It was formerly believed that thyrotrophic hormone on the thyroid produces its effect directly through the blood-stream and not by a



nervous mechanism [9]. Recent investigations [10, 11], however, have shown that under certain conditions the function of the thyroid gland depends partly on its nerve supply. In the pregnant and pseudo-pregnant rabbit, the denervation of the thyroid gland by means of autotransplantation reduces basal metabolism, or retards its increase except during the last third of pregnancy [10]. In an extensive study on the autotransplantation of the thyroid gland in 175 rats, 11 rabbits and 3 guinea pigs, it was demonstrated that survival of the transplanted gland depends on the ability of its nerve supply to regenerate immediately following transplantation. A greater percentage of survivals was obtained in animals not older than 12-14 days [11].

#### STANDARDIZATION

The commercial hormones are standardized by their ability to raise the basal metabolism of hypophysectomized rats and guinea pigs, and also by the changes they produce in the thyroid glands of these animals.

An international standard is not available. Thyrogan, a commercial preparation of thyrotrophic hormone, for example, is standardized in guinea-pig units, each ampoule containing 50 guinea-pig weight units, a unit being that amount of Thyrogan which, given in a series of five injections, will double the weight of the thyroid of a batch of immature guinea pigs in 5 days.

#### ADMINISTRATION

Intramuscular injections.

#### DOSAGE

Fifty guinea-pig weight units 2-4 times weekly according to the grade of thyroid hypofunction.

Investigations carried out with the thyrotrophic hormone in cases of myxoedema resulted in a rise of basal metabolism when myxoedema was due to pituitary failure. Reports on the thyrotrophic hormone exerting a beneficial effect in only a small percentage of cases with myxoedema [12, 13, 14] are probably due to the fact that these non-responsive cases were characterized by a non-functioning thyroid gland of primary cause. Sharpey-Schafer and Schrire [153], on the other hand, observed cases which were resistant to the stimulus of the thyrotrophic hormone, although they had functioning thyroid glands. Non-myxoedematous patients suffering from a low basal metabolic rate due to insufficiency of the pituitary usually benefit by the administration of thyrotrophic hormone.

## CLINICAL APPLICATION

The thyrotrophic hormone has not been extensively tried in man.

Greene [7] reported beneficial results with the thyrotrophic hormone in patients suffering from cold extremities due to arterial spasms. He introduced the term "hypothyrokinesis", denoting hypothyroidism due to failure of the thyrotrophic hormone.

## ADRENOCORTICOTROPIC HORMONE (ACTH)

Corticotrophic hormone is prepared from the anterior lobe of animal pituitary glands. There are no synthetic or crystalline preparations available. The commercial preparations usually contain an admixture of growth, thyrotrophic and lactogenic hormones.

## PHYSIOLOGY

The anterior pituitary elaborates ACTH apparently at a rate inversely proportional to the concentration of cortical hormone(s) in the body fluids and according to the requirements of the peripheral tissue cells for cortical steroids [165].

Adrenal cortical hormone, administered immediately before exposure of the rat to any one of a number of stresses (cold, heat, injection of histamine, epinephrine, or killed typhoid organisms), suppresses the enhanced adrenal cortical activity which otherwise follows such an exposure. The data suggest, but do not offer conclusive proof, that the cortical steroids act directly on the anterior pituitary rather than indirectly through products of their metabolic activity or their deficiency. Castration of the male animal is followed by adrenal hypertrophy [19] which is inhibited by male sex hormone.

Injections of ACTH into normal male rats decrease adrenal ascorbic acid concentrations, and experimental findings have demonstrated a quantitative relationship between ACTH dosage and adrenal ascorbic acid response [47, 116].

The rate of delivery of lymphocytes to the circulation is partly controlled by ACTH secretion. The lymphopenic effect of adrenal cortical steroids is a reflection of lymphocytic dissolution in lymphoid organs. Conversely, the lymphocytosis of adrenalectomized animals is probably due to the absence of the hormonal mechanism normally controlling lymphocytic dissolution [169, 113].

In normal human beings ACTH administration induces an increase in neutrophils, a decrease in lymphocytes and a decrease in eosinophils; whereas similar treatment in patients with Addison's

disease is accompanied by only a slight increase in neutrophils without a significant change in lymphocytes or eosinophils [110]. In rats with persistent diabetes mellitus induced by alloxan, ACTH increases the degree of glycosuria and nitrogen excretion both with and without exogenous insulin administration [167, 168, 112].

The function of the adrenal cortex depends on stimulation not only by the ACTH of the anterior lobe of the pituitary (see Chapter V), but also by a diversity of other factors. Stimuli which may cause adrenal cortical enlargement are the following:

Oxygen deficiency.

Chronic cyanide poisoning.

Thiamine deficiency.

Exposure to cold.

Physical exercise.

Factors causing increased breakdown of proteins, viz.:

Injection of peptone [15]

Feeding of proteins [16, 17]

Inanition (the adrenal cortex atrophies in chronic sub-nutrition).

Toxic Substances, such as alkaloids, heavy metals, phenol derivatives, formaldehyde, ammonia and bacterial toxins.

Hormonal Factors :

Oestrogens, thyroid, insulin.

### **Adrenal Cortical Autonomy**

The adrenal cortex is characterized by its relative independence of the pituitary gland. This is shown, for example, by its function under the influence of insulin, thyroid and small doses of oestrogen after hypophysectomy; and also by the fact that the hypophysectomized animal does not exhibit evidence of total adrenal insufficiency. According to Goldzieher [23] adrenal cortical insufficiency, in experimental animals and humans, mainly affects carbohydrate and protein metabolism, whereas, in the hypophysectomized animal and in advanced stages of Simmonds's disease, only slight changes and disturbances in vascular permeability and electrolyte metabolism have been noted.

According to this author, the corticotrophic factor of the anterior lobe is, however, essential for the response of the adrenal cortex to almost all stimuli. Its effect is markedly augmented by addition of a dilute suspension of pituitary tissue [25].



Injections of oestrogen in moderate doses maintain the size of the cortex in the hypophysectomized rat. In the intact animal, oestrogens in moderate dosage produce considerable hypertrophy of the cortex, whereas excessive doses are less effective or may even cause degenerative changes [18]. The cortical hypertrophy produced by oestrogens is prevented by hypophysectomy [88].

Castration of the male animal is followed by adrenal hypertrophy [19] which is inhibited by male sex hormone. Cortical extract, desoxycorticosterone acetate, and a unilateral cortical tumour inhibit pituitary corticotrophic activity [23].

### **Relation to Thyroid Hormone**

Thyroxin causes adrenal enlargement [20], whereas thyroidectomy inhibits the response of the adrenal cortex even to injections of pituitary extracts [21]. The cortical hypertrophy produced by administration of thyroid substances is actually expressed by increased function [22].

### **Relation to Insulin**

Prolonged administration of insulin produces adrenal hypertrophy (even in the hypophysectomized pigeon).

### **Relation to Growth Hormones**

The corticotrophic hormone, in its action upon the adrenal cortex, is markedly antagonistic to the growth hormone. Administration of corticotrophic hormone inhibits growth in both the normal and gonadectomized male rat but not in the adrenalectomized rat [26, 27]. The hormone acts in the normal rat by retardation of chondrogenesis and osteogenesis in the region of the proximal epiphysis of the tibia [27].

The action of the pituitary growth-hormone, in the female hypophysectomized rat, is inhibited by simultaneous administration of pituitary corticotrophic hormone [28]. The animals gain weight at about half-rate, in contrast with those injected with growth hormone alone [29]. On the other hand, hypophysectomized rats which are also adrenalectomized show no greater response to the growth hormone than rats which are hypophysectomized only [30].

### **Relation to Thymus**

The inhibiting action of the thymus on acetylcholine synthesis [31], necessary for the transmission of nervous impulses, is removed by administration of corticotrophic hormone. The brains of mice, treated

with corticotrophin for 15 days, synthesized on the average 50 per cent more acetylcholine than did those of control animals [32] (see Chapters V and X).

ACTH 2.5 mg. given every 6 hours for 4 doses produced hypertrophy of the adrenal glands and atrophy of the thymus. The zona fasciculata of the adrenal cortex showed a noticeable decline in birefringence and sudanophilia. This observation indicates that the zona fasciculata of the adrenal cortex is under pituitary control and secretes steroid hormones which influence thymus size and glycogen deposition in the liver [111].

#### STANDARDIZATION

The Sudanophobic Unit is defined as the total injectable dose which, divided into eight intraperitoneal injections given twice daily on each of four successive days, causes disappearance of the Sudanophobic zone and reconstitution of a normal lipoid content of the adrenal cortex of the male rat, hypophysectomized 7–10 days before the first injection, and weighing between 100–150 grams.

#### CLINICAL APPLICATION

Hemphill and Reiss [33, 34, 35] treated successfully with corticotrophic hormone a number of cases of anorexia nervosa and severe melancholic depression, associated with clinical pituitary cachexia (emaciation, asthenia, loss of hair, desiccated skin and low output of 17-ketosteroids) which developed after the menopause. These authors [36] recently reported a case of clinical pituitary cachexia in a nulliparous woman—clinical and hormonal studies confirming extreme hypopituitarism. Two courses of corticotrophic hormone, the first consisting of 40 Sudanophobic Units daily for 24 days, and the second, 2 months later, of a further course of 14 daily injections of 25 units, resulted in complete restoration of weight and cosmetic features. The authors stress the point that this was not a case of anorexia nervosa. The psychotic depression was only removed a considerable time after physical improvement was complete.

#### LACTOGENIC HORMONE (PROLACTIN, LUTEOTROPHIN)

Lactogenic hormone is extracted from anterior lobes of animal pituitaries. The hormone is more plentiful in some species and at certain periods of life than in others. Beef and sheep glands usually yield 30–40 I.U. per grain of fresh tissue.

## PHYSIOLOGY

The lactogenic factor, seemingly produced in the eosinophilic cells of the anterior pituitary [37], is the regulator of lactation but not of breast development. It produces gonadal atrophy in adult birds [38], initiates the incubation instinct in fowls [39] and causes growth of the crop glands and an increase in weight and appetite in pigeons. In some mammals it causes an increase in the basal metabolism [40].

In the human subject the only effects of the hormone appears to be lactogenesis and the control of some types of uterine bleeding.

The name "luteotrophin" has often been applied to the lactogenic hormone since it was used in cases of functional uterine bleeding. This term is slightly confusing, for prolactin does not possess luteinizing properties, although its action on the ovary is luteotrophic. It can stimulate luteal tissue to produce more progesterone after the luteinizing hormone of the anterior pituitary gland has brought about development of the corpora lutea. Prolactin does not possess the power to initiate the luteinization of the follicles. Therefore it is ineffective in non-ovulatory cycles or in conditions in the male in which an interstitial cell stimulating effect is desired.

## RELATION TO OTHER GLANDS

**Growth**

The lactogenic hormone has a synergic effect on growth when administered together with the thyrotrophic hormone [41].

**Oestrogens**

Oestrogens increase the amount of lactogenic hormone in the pituitary gland of the rat [42, 169-71], guinea pig [171, 142] and rabbit [173] from 200 to 500 per cent. From a cytological point of view it is interesting to note that oestrogens have been shown to increase the number and secretory activity of the eosinophilic cells of the anterior pituitary [174-7], which are agreed to be the source of lactogenic hormone in both mammals [137, 145, 146] and pigeons [136]. The ability of sex hormones, particularly oestrogens, to increase lactogen in the anterior pituitary is also reflected in the greater quantities of lactogenic hormone found in the blood of rabbits after oestrone administration [173] and in the initiation of milk secretion in a number of mammals following oestrogen administration [142, 135]. The authors have advanced the theory that oestrogen is the factor normally responsible for stimulating the secretion of pituitary



lactogen and initiating milk secretion at the time of parturition [142] (see Chapter VII).

### Thyroid

The thyroid hormone is another agent with well-marked galactopoietic effect in the lactating cow and goat [43, 44].

#### STANDARDIZATION

The lactogenic hormone is standardized in bird units based on an increase in weight of pigeons' 'crop glands. Samples of a standard preparation of the lactogenic crop-gland-stimulating hormone are available from the National Institute for Medical Research, Hampstead, London. The International Unit is defined in terms of the standard preparation.

#### ADMINISTRATION

Intramuscular injections.

#### PREPARATION FOR CLINICAL USE

Prolactin: Ampoules 1 c.c. containing 100 I.U.

Range of single dose: up to 200 I.U.

Range of total dose: up to 2,250 I.U.

#### CLINICAL APPLICATIONS AND APPROXIMATE DOSAGES

Lactogenesis involves several hormones in addition to many other factors and, on the whole, it seems that the lactogenic hormone can only initiate milk secretion in prepared mammary tissue.

### Deficient Lactation

Total dose:

900 I.U. intramuscularly to be administered as follows:

150 I.U. twice daily on the first and second days.

60 I.U. twice daily third and fourth days.

60 I.U. once on the fifth day [45]

or

100 I.U. twice daily from the first through the fifth postpartum day.

In an experimental series more than twice as much milk was obtained from treated women than from untreated controls [46].

### Functional Uterine Bleeding

The recommended dosage in menorrhagia is 200 units daily until bleeding stops but at least for 4 days starting at the onset of menstruation; thereafter every other day for at least 1 week; repeated for 2-3 months until normal cycles are established.

Only a few clinical reports are available [132-4], but although these record beneficial results, the rationale for the use of prolactin is not quite clear and it is doubtful whether it is superior in effect to other haemostatic measures employed for excessive functional bleeding.

### GONADOTROPHIC HORMONES

The gonadotrophin preparations in clinical use are obtained mainly from three sources: the anterior lobes of pituitary glands, the urine of pregnant women, and the serum of pregnant mares. They are termed accordingly:

1. Pituitary Gonadotrophic Hormone (from the anterior lobes of pituitary glands).
2. Chorionic Gonadotrophic Hormone (from the urine of pregnant women).
3. Equine Gonadotrophic Hormone (from pregnant mares' serum).
4. Anterior Pituitary Hormone combined with Chorionic Gonadotrophic Hormone (contains minimal amounts of anterior pituitary gonadotrophic hormone plus chorionic gonadotrophin).

The gonadotrophic hormones have not yet been synthesized; but they have recently been obtained in almost pure form. Chorionic gonadotrophin has predominantly luteinizing properties; equine gonadotrophic hormone exerts, in the laboratory animal, both follicle-stimulating and luteinizing effects, whereas, in the monkey and human being, it has a primarily follicle-stimulating effect.

### PHYSIOLOGY

#### 1. Pituitary Gonadotrophic Hormone

The number of the gonadotrophic factors elaborated by the anterior lobe of the pituitary gland has for long been a matter of controversy. There is evidence for the view that it elaborates two gonadotrophic factors—the follicle-stimulating (FSH) and the luteinizing (LH) hormones.

Contrary to earlier beliefs that, in the menstrual cycle, the follicle-stimulating hormone induces ripening of the follicles during the first fortnight, and the luteinizing factor produces and maintains the corpus luteum during the second half of the cycle, it is now held that both hormones are released by the anterior pituitary gland simultaneously in varying amounts. According to Severinghaus [1] FSH is produced by the basophil cells and the LH by the eosinophils. This was recently confirmed by the experimental evidence of Baker and Everett [97]. They demonstrated that diethylstilboestrol, injected into 25-day-old rats in daily doses ranging from 0.05 to 4.0  $\mu\text{g.}$  for 4-7 days, causes increase in the relative and absolute number of eosinophils, with increased mitotic activity and hypertrophy of the golgi apparatus. The basophils show degranulation and reduction in numbers. The relative and absolute numbers of chromophobes are reduced with little change in their mitotic activity. The total weight of the pituitary is increased. Injection of the smaller dose of 0.05  $\mu\text{g.}$  daily for 14 days, however, resulted in a slight reduction in the number of eosinophils and increase in the number of chromophobes. The authors conclude that oestrogen exerts a stimulative action on the eosinophils; and since oestrogenic activity induces release of LH from the anterior lobe [140-4], it is assumed that the eosinophils are the source of LH.

Further evidence has recently been produced in support of the theory that FSH is produced by the basophils and LH by the eosinophils. The injection of oestradiol reduces the percentage of basophils in the pituitary glands of rats and at the same time increases the number of eosinophils. This suggests that the differentiation of each type of chromophils from chromophobes is controlled by the level of blood-oestrogen [131]. Others [130] report that the pituitaries of spayed animals subsequently treated with oestradiol benzoate have produced a high degree of follicular activity but no corpora lutea, indicating removal of the luteinizing factor by oestrogens. The different effects on the pituitary obtained with oestrogens by various workers is apparently due to the different dosages employed.

In the regularly menstruating woman, a peak of gonadotrophin excretion consisting of both fractions occurs at the mid-cycle prior to ovulation. The amounts of gonadotrophin excreted during the peak period vary from 2 to 25 rat units per litre of urine [48, 49, 50]. This is preceded by a peak of oestrogen excretion, reaching 1,000 I.U. per 24 hours-urine [50]. A second peak of gonadotrophin excretion immediately before menstruation has been postulated, but the evidence is not convincing. Some observers [51] maintain that there is no second



peak, suggesting that the pituitary stimulating effect of the second oestrogen peak is inhibited by progestin.

The recent preparation of highly purified pituitary extracts of both gonadotrophic factors [52-9] has shown that, although follicular growth occurs under the influence of FSH, no oestrogen is produced unless LH is injected simultaneously [60]. The same applies to ovulation. This does not occur unless both hormones, FSH and LH, act simultaneously and in a definite ratio [60-3]. This combined action coincides with a particular stage of follicle maturation, being the point at which it is ripe for rupture. Experimental evidence [61] suggests that once this point is passed it can no longer respond to an ovulating stimulus.

Normal pituitary gonadotrophic function is presumably under the control of the hypothalamus and depends on impulses leading through the hypothalamic-pituitary nervous pathways [64]. In particular, the intact connection between the pars tuberalis and the pars distalis is a pre-requisite of normal production of gonadotrophic hormones. The impulses which pass from the hypothalamus through the infundibulum and the processus infundibuli are of no significance for sexual function [65]. In addition, normal gonadotrophic function depends on a balanced inter-relationship of the pituitary with other hormones, and particularly those of the ovary in the woman.

In the normally menstruating woman, the combined action of FSH and LH produces follicle growth with oestrogen secretion. Oestrogen secreted by the theca interna evokes the differentiation of the granulosa cell layer [129] and stimulates the functional activity of the basophils of the anterior pituitary (increase in mitochondria and golgi apparatus and degranulation), which release the FSH [24, 58, 69, 132-7]. Under the action of this gonadotrophin, further follicular development occurs, with increasing concentration of oestrogen. Increased oestrogenic activity stimulates, in addition, the eosinophils of the anterior pituitary which produce the LH and by this means induces ovulation and luteinization of the granulosa cells with production of progestin. Others [66, 67] believe that the increased output of LH, caused by the excess of oestrogen, is associated with a decreased release of FSH.

Progestin, in turn, activates the eosinophils, which then elaborate additional luteinizing gonadotrophin or perhaps a third gonadotrophin the luteotrophic hormone, which is identical with the lactogenic factor and distinct from LH since it is concerned solely with the maintenance of a formed corpus luteum and is not able to induce ovulation. In addition to progestogen, increasing amounts of

oestrogen are produced by the corpus luteum. The peak of progesterone concentration again initiates basophil cell activity with the release of FSH [1]. At this point there occurs regression of the corpus luteum, connected with a marked drop in the oestrogen and progesterone

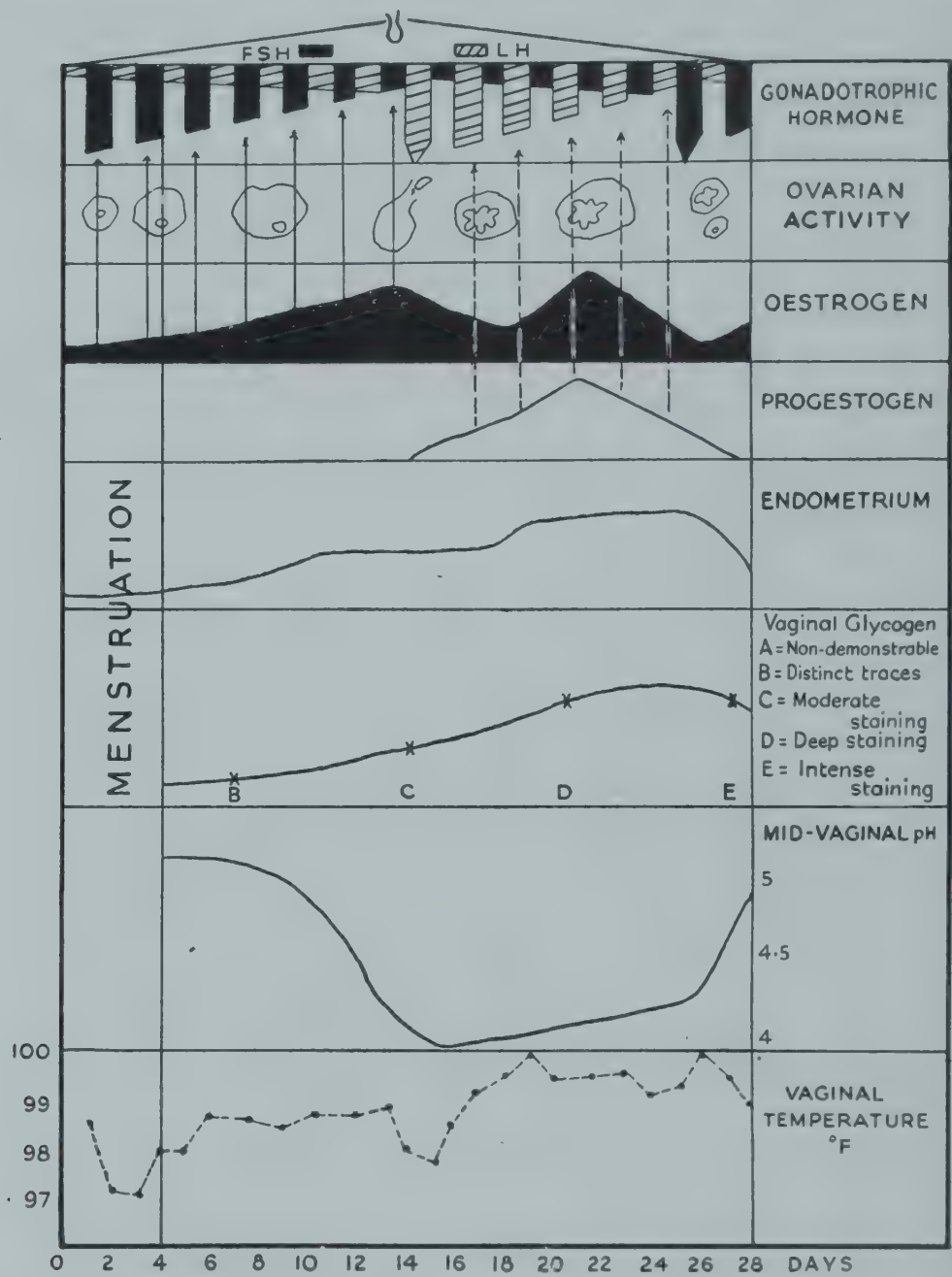


FIG. 3.—THE MENSTRUAL CYCLE

togen level. It is suggested that the renewed activity of the basophilic cells is due to the excess of progesterone inhibiting the output of LH or luteotrophin, thus causing corpus luteum regression [70] (see Figs. 2 and 3). Endometrial disintegration occurring at about this stage

of the pituitary cycle marks the onset of a new menstrual cycle (see Chapters VII and VIII).

In the male, the follicle-stimulating hormone stimulates spermatogenesis (gametokinetic factor), and the luteinizing hormone acts on the interstitial cells of the testes producing male hormone (interstitial-cell-stimulating hormone, ICSH).

It has frequently been suggested that pituitary gonadotrophic activity in the male follows a cycle similar to that in the woman. This hypothesis, however, is not supported by any evidence (see Chapter IX).

### *Assay Methods*

The assay of gonadotrophic hormones is based on biological response since no chemical methods are available.

Various methods are described.

1. Seminal vesicle weight increase.
2. Ovarian weight increase.
3. Uterine weight increase.
4. Luteinization.
5. Vaginal cornification.

## **2. Chorionic Gonadotrophic Hormone**

This hormone, first discovered by Aschheim and Zondek, is extracted from the urine of pregnant women. It appears in the blood and urine soon after implantation of the ovum and comes from the chorionic elements of the placenta. The concentration of hormone in the blood and urine increases rapidly during the first part of pregnancy, reaching its peak between 50–60 days from the first day of the last menstrual period. Thereafter, it gradually declines to relatively low levels which are maintained until a few days after parturition, when the hormone entirely disappears from the blood and urine. Chorionic gonadotrophin has also been demonstrated in the blood and urine of women with hydatid mole and chorionepithelioma and of men with embryonic testicular neoplasm.

In the female the gonadotrophin from human pregnancy urine has predominantly luteinizing property, while in the male it primarily stimulates the interstitial cells, and has no effect on spermatogenesis.

In the female rat, chorionic gonadotrophin causes follicle growth and formation of corpus luteum; and it produces a prolonged oestrus [hypophysectomy is performed after corpus luteum formation [71, 72]. The stimulant effect of this hormone on ovarian weight is



considerably less marked than that of the pituitary gonadotrophin [138]. Its action, however, is greatly augmented by simultaneous injections of stilboestrol. The ovaries of immature female rats thus treated show development of fairly large follicles and corpora lutea with a marked increase in ovarian weight [139, 73, 74].

In the woman human chorionic gonadotrophin is luteotrophic and will induce a pseudo-pregnant condition, as shown by a prolongation of the functional life of the corpus luteum, the development of a decidua and the prolonged excretion of pregnanediol. The effective dose of chorionic gonadotrophin seems to be 5,000 I.U. to 10,000 I.U. daily. These doses may be given without untoward reactions [128]. The negative results in an earlier report [75] were undoubtedly due to the low doses employed.

### 3. Equine Gonadotrophic Hormone

This hormone appears in large quantities in the blood of pregnant mares during the early stages of gestation, reaching a very high concentration at the middle third of pregnancy. Opinions are divided as to whether this hormone is of pituitary or chorionic origin. The fact that its action is primarily follicle-stimulating suggests a pituitary origin. Catchpole and Lyons [76], however, have observed that the hormone accumulates predominantly in the fertile horn of the uterus, and especially at the site of the chorionic vesicles. A peculiarity of this hormone is that it is not excreted, and thus can at no time be demonstrated in the urine of the pregnant mare, or of animals treated with this hormone. It is suggested that this is due to inability of the hormone to pass the kidney.

Evans and others [77] demonstrated, by experiments in the hypophysectomized female rat, that equine gonadotrophin consists of two fractions, one having a follicle-stimulating, and the other a luteinizing effect. In intact immature female rats, pregnant mares' serum produces follicular stimulation with subsequent luteinization and marked increase in ovarian weight. In the hypophysectomized adult male rat the equine hormone is able to maintain spermatogenesis and thus produce fertile matings [78].

Evidence of its beneficial effect in the human subject is very contradictory.

### 4. Gonadotrophic Synergism

In 1931 Evans and others [79] combined a crude anterior pituitary extract with chorionic gonadotrophin and found that the resulting gonadotrophic potency was greater than could be ascribed to the

mere addition of the two factors. He accordingly postulated a synergic principle in the anterior pituitary gland. Fevold and his group [80, 81] proved that the phenomenon was due to a synergic interaction of the follicle-stimulating and luteinizing factors. They observed that purified follicle-stimulating and luteinizing extracts of the anterior pituitary, when re-combined, produced an increase in ovarian weight far exceeding that obtained from the two factors given singly.

Non-specific materials, for example, tannic acid, copper, zinc sulphate, casein or egg albumen, extract of liver, milk, lemon juice or yeast, are capable of augmenting the potency of gonadotrophic preparations, and on these grounds some workers ascribe the synergic action of the pituitary follicle-stimulating hormone to its protein nature. It has been shown, however, that when the pituitary extract is inactivated by heating, or replaced by muscle extract having the same protein content, the augmentation phenomenon of the gonadotrophic hormone does not occur [82].

#### STANDARDIZATION

##### **1. Anterior Pituitary Gonadotrophic Hormone (from the Anterior Pituitary Gland of Sheep and Cattle)**

No international standard has yet been established. Preparations are assayed on the basis of ovarian and uterine response in the immature rat. The unit represents the degree of response and varies with each manufacturer.

##### **2. Chorionic Gonadotrophic Hormone**

An International Unit was established by the Health Organization of the League of Nations in 1939. The International Unit is equivalent to the gonadotrophic activity of 0.1 mg. (100 gamma) of a standard dry powder prepared by members of the conference. It represents approximately the amount required to produce vaginal cornification in the immature rat.

##### **3. Equine Gonadotrophic Hormone**

The International Unit (I.U.) is defined as a specific gonadotrophic activity of 0.25 mg. (250 gamma) of the standard preparation held by the Health Organization of the League of Nations.

This hormone was first standardized in rat units (Cole and Saunders)—a "Cole-Saunders" Rat Unit representing the amount of hormone which, after a single subcutaneous injection into 5 immature 1-23-day-old female rats, will produce an average of 3-10 large

follicles or corpora lutea at the end of 96 hours and which, if halved, will not produce this average in 5 rats similarly treated.

The "Cartland-Nelson" Rat Unit is the amount of hormone which, after several daily subcutaneous injections to 21-23-day-old female rats weighing 30-40 grams, will produce in 5 days a pair of ovaries weighing 65 mg., whereas the ovarian weight of the uninjected controls is 13 mg.

- 1 "Cartland-Nelson" Unit equals approximately 10 "Cole-Saunders" Units.
- 1 "Cole-Saunders" Unit equals approximately 2 International Units.
- 1 International Unit equals approximately  $\frac{1}{20}$  "Cartland-Nelson" Unit.

#### 4. The Anterior Pituitary combined with Chorionic Gonadotrophic Hormone (Synapoidin)

This combination has been standardized in synergy units, a unit representing the minimum quantity of the substance which, when injected subcutaneously into 28-day-old immature female rats in 6 divided doses over a period of 3 days, produces an ovarian weight 5-6 times that of the untreated animals.

- 1 Synergy Unit is equivalent to 1 "Cartland-Nelson" Rat Unit  
*and*

- 15 "Cartland-Nelson" Units are contained in 1 c.c. of the substance.

#### ADMINISTRATION

The gonadotrophic hormones are administered intramuscularly; the gonadotrophin from pregnant mares' serum can, in addition, be injected intravenously.

#### PREPARATIONS FOR CLINICAL USE

##### 1. Pituitary Gonadotrophic Hormone

A large number of anterior lobe preparations are available, but there is no evidence that all such preparations are of value in treatment. Experimental preparations have been produced in which the active principles have been isolated in relatively pure form and which have shown great potency in animal experiments.

Davis and Hellbaum [83] recently recorded their results with fractionated and unfractionated gonadotrophic hormones of sheep



pituitary. The extracts were prepared and provided by R. K. Meyer and W. H. McChan of the University of Wisconsin. Although both the fractionated and unfractionated hormones produced ovarian stimulation in the human, the latter proved the more potent preparation. It was observed and shown by laparotomies that the ovaries of the adult woman in the reproductive period, during pregnancy, and in the early puerperium respond to gonadotrophic stimulation. Follicle growth of all degrees was induced, and possibly, in several instances, follicle rupture, but in no case corpus luteum formation.

Recently a pituitary gonadotrophic preparation from horse pituitaries has been made available in the U.S.A. and has shown promising results. Although frequently referred to as "equine gonadotrophin", it is distinct from preparations made from pregnant mares' serum. It is said to be a highly purified stable preparation extracted from the pituitary gland of horses, the glands of which are richer in gonadotrophin than those of any other species except man. It contains the follicle-stimulating and luteinizing hormones, the former being predominant and the latter present in small amounts.

This equine gonadotrophin has been used in a diversity of conditions involving ovarian failure as a result of pituitary hypofunction: e.g. primary and secondary amenorrhoea [119-22], menorrhagia [119, 122], irregular and infrequent menstruation [119-22], infantilism [120, 121], and sterility [119, 120, 122]. In the male it has been used to treat underdeveloped testes and genitalia [122] and Fröhlich's syndrome [121]. Beneficial results are reported. It appears to have a distinct stimulating action on the human gonad but also elicits anti-hormone formation [117].

## 2. Chorionic Gonadotrophic Hormone

Although chorionic gonadotrophin has proved successful in inducing follicular maturation, ovulation and corpus luteum formation in the animal, beneficial results in the human have been obtained only in two instances: (a) cryptorchidism, and (b) by cyclic treatment following the administration of pregnant mares' serum in cases of menstrual disorder and sterility not associated with hyperoestrogenism [84, 85, 98].

Hamblen [86] showed that chorionic gonadotrophin, when given alone, does not stimulate ovarian function but, on the contrary, tends to promote follicular atresia. An existing corpus luteum, however, responds to chorionic gonadotrophin by increased function [86, 87]. Rydberg and Pedersen-Bjergaard [84] confirmed the results of Hamblen, but found that the hormone produced successful luteiniza-

tion if treatment was preceded by intramuscular injections of equine gonadotrophin. Using a method of combined pregnant mares' serum and chorionic gonadotrophin routine treatment, these authors were able to restore ovarian function in a majority of cases of secondary amenorrhoea and, in some cases, of primary amenorrhoea.

Gordon and Fields [89, 115] found chorionic gonadotrophin of great value in the treatment of cryptorchidism, pre-adolescent short stature and hypogenitalism; better results, however, were obtained when treatment was alternated with injections of testosterone propionate. The results obtained in cryptorchidism are due to the stimulation of the interstitial cells causing increase in the size of the testes. Increased male hormone production is also responsible for the acceleration of growth in cases of delayed puberty [90].

### 3. Equine Gonadotrophic Hormone

Prepared from the serum of pregnant mares, this hormone has a follicle-stimulating effect in the female and a spermatogenic action in the male animal. There is much doubt whether, in the human, this hormone, when given alone, is capable of inducing ovulation or producing spermatogenesis. Indeed, there is growing evidence that equine gonadotrophin, beyond slight follicular stimulation, is not capable of inducing ovulation or corpus luteum formation.

Davis and Koff [91] were apparently the first to succeed in inducing ovulation in a series of patients—i.e. 16 out of 36. The women, however, were menstruating normally. Siegler reports that he was able to induce ovulation with equine gonadotrophin treatment. He gave 300 I.U. to women with non-ovulatory menstrual cycles. The number of successfully treated patients was 16 out of 30 [92]. The evaluation of these results is difficult, since non-ovulatory and ovulatory cycles frequently alternate. Griffiths and McBride [93] recorded ovulation in 7 out of 8 patients with a total dose of 800–1,100 I.U. in two successive doses from the ninth to the fourteenth day of the cycle. These patients had non-ovulatory cycles, however, and the results should therefore be treated with the same reserve.

Irving, Sears and Rock [94], in a series of cases investigated by endometrial biopsy, found no definite evidence that equine gonadotrophin had stimulated ovulation. Brewer, Jones and Skiles [95] used total doses of 600–5,000 Cole-Saunders Units of pregnant mares' serum. Inspecting the ovaries on laparotomy, they found no evidence of beneficial results. Geist, Gaines and Salmon [96] examined the ovaries of 91 women but in no case could relate ovulation to equine gonadotrophin treatment. Although Hamblen [86] ascribed some



stimulation of follicles to such treatment, no ovulation as shown by endometrial biopsies has been observed. Rydberg and Pedersen-Bjergaard [84], injecting equine gonadotrophin intravenously, obtained maturation of follicles but no ovulation.

Results incomparably better than those obtained with any other form of gonadotrophic hormone therapy have been obtained by cyclic administration of equine gonadotrophine [85, 98–100]. Rydberg and Pedersen-Bjergaard [84] have recorded striking results with cyclic administration of high doses of equine gonadotrophin and chorionic gonadotrophin.

### *Spermatogenesis*

Equine gonadotrophin treatment has proved ineffective in azo-spermia and oligospermia—producing no improvement in volume of semen, number of spermatozoa, morphology or immediate motility. Treatment with pituitary, chorionic and equine gonadotrophin singly, with pituitary and chorionic gonadotrophin combined, or with equine gonadotrophin and testosterone propionate combined, has shown no beneficial results [101].

Charny [102] reviews his investigations in 127 cases of deficient spermatogenesis. Correlating his investigations with sperm counts and testicular biopsies, he found that 48 of the patients—a surprisingly high percentage (37·8)—had irreparable lesions of the seminiferous tubules, the most prominent of which were peri-tubular fibrosis. Gonadotrophin therapy in these cases is obviously useless. Seventy-nine of the patients (62·2 per cent) had seminiferous tubules which could conceivably regenerate. These 79 patients were treated with equine gonadotrophin in doses of from 400 I.U. to 2,000 I.U. intravenously, 3 times weekly for a period of 3–6 months. Twelve patients (15·3 per cent) were cured with a resultant total sperm count of at least 200 millions. Twenty-two patients (27·8 per cent) improved, showing an increase in sperm count of at least several millions. The remaining 45 patients (57 per cent) showed no change in sperm count after treatment. Testicular biopsies were repeated after a test period of treatment in 15 of these patients. Of these 11 (73 per cent) revealed increased growth of the germinal epithelium of the tubules, although the semen picture remained unchanged.

The author suggests that failure to obtain beneficial results might have been due to the fact that the administered gonadotrophin was not sufficiently potent and should have been administered in larger doses.



#### 4. Anterior Pituitary Hormone combined with Chorionic Gonadotrophin (Synapoidin)

Mazer and Ravetz [103] reported their experiments with Synapoidin, a combination of anterior pituitary extract and chorionic gonadotrophin. In 19 out of 23 patients suffering from amenorrhoea, one or more menstrual flows followed treatment with this combination. Examination of the ovaries following treatment with varying doses of the preparation revealed over-sized ovaries, haemorrhagic follicles and, in many instances, multiple, incompletely-formed corpora lutea. Abnormal uterine bleeding was controlled in 14 out of 18 patients. Two out of 8 women suffering from sterility associated with non-ovular menstruation conceived shortly after treatment.

Mazer and Israel [104] reported in 5 patients restoration of menstrual periodicity which continued after cessation of treatment; and in 27 out of a total of 35 patients suffering from amenorrhoea evoked menstrual bleeding during the period of treatment only. Nine patients, before operation for uterine fibroids and unilateral ovarian cysts, received Synapoidin 2-3 c.c. daily or every other day for from 2 to 9 days. On operation, 8 of the 9 patients showed haemorrhagic follicles of large size, some of them reaching the size of a hen's egg. In those patients in whom oophorectomy was permissible because of age, multiple corpora lutea were demonstrated in one or both ovaries.

The authors maintain that, in the human subject, the combination of chorionic gonadotrophin and one of the pituitary extracts evokes a degree of ovarian activity not obtainable with either of the two employed individually. Geist, Gaines and Salmon [105], however, found that the degree of stimulation from the combined pituitary and chorionic gonadotrophins was not greater than when the pituitary extracts were injected alone.

Davis [106] treated 27 cases of amenorrhoea with Synapoidin. In all cases the dosage was 1 c.c. intramuscularly 3 times weekly for 3 weeks, provided that haemorrhage did not supervene or that the ovary did not enlarge unduly. Smaller doses were found to be relatively useless. Before treatment patients were subjected to careful examination in order to find the precise cause of amenorrhoea and to group them accordingly. In 19 cases, the amenorrhoea was of primary pituitary origin; in 6 it was the direct result of ovarian destruction, and in 2 the thyroid was at fault. No allergic reactions were noticed. Excessive bleeding occurred in 3 cases but was repeated spontaneously only in one. In 6 of the patients enlargement of the

ovaries occurred. Growth was usually rapid and often considerable, the ovary in one of the cases developing to the size of a large lemon in 6 weeks. The involution was correspondingly slow, and it usually took several months for the organ to resume its normal size. Fourteen out of the 27 patients showed an apparently permanent cure (restoration of normal menstrual rhythm for at least 6 months); 6 patients showed temporary improvement, and the remaining 7 did not benefit at all by the treatment.

It was found that Synapoidin therapy was most effective in the mild Fröhlich hypopituitary type of patient, particularly when increase in weight was fairly marked (when obesity was absent, the results were usually poor). The 6 cases which showed only temporary improvement did not menstruate spontaneously, but bleeding could be induced repeatedly by Synapoidin injections.

Hoffman [107] advises great caution in the use of Synapoidin, since overdosage may cause extreme ovarian enlargement accompanied by low abdominal pain, nausea, vomiting and even fever. He suggests that this may be due to intraperitoneal haemorrhage from the large haemorrhagic follicles found in such overstimulated ovaries. Allergic shock has also been reported after treatment with Synapoidin.

Davis and others [108] used the combined anterior pituitary and chorionic gonadotrophic hormone for the treatment of seminal inadequacy in 20 males aged from 24 to 25. Thirty to forty Synergy Units, intramuscularly, were administered for 4–12 weeks. The average duration of a course of treatment was 5–7 weeks and the average series dosage 1,300 Synergy Units. The seminal values showed some increase in 8 cases, decrease in 5, and no change in 7. Post-treatment values, however, showed a trend towards lower levels than pre-treatment ones. The authors suggest that antibody formation may have occurred.

#### SUMMARY

The use of the gonadotrophin preparations at present available has yielded many disappointing results. This, however, does not prove their ineffectiveness, since the most potent substance may fail to produce positive results if unphysiologically applied. In order to secure satisfactory results in the treatment of endocrine disorders, a thorough understanding of glandular physio-pathology and of the physiological and pharmacodynamical action of the preparation employed is necessary. Any estimation of the worth of data purporting to show the effectiveness or otherwise of gonadotrophin preparations

must take account of the extent to which this basic principle of endocrine practice has been followed.

The chorionic and equine gonadotrophic hormones, administered alone, have proved thoroughly ineffective in the female. Although there seems to be ample proof that pregnant mares' serum stimulates maturation of follicles, there is no evidence that it can induce ovulation. The combined anterior pituitary and chorionic gonadotrophin preparation has given somewhat contradictory results, and its evaluation, therefore, must be postponed until more convincing evidence is available. It seems that the best results have been those obtained by Hamblen's so-called 1-2 procedure, consisting of equine gonadotrophin injections for 10 days starting on the fourth day of the cycle, followed by chorionic gonadotrophin for the next 10 days, and by the Rydberg and Pedersen-Bjergaard method of administering 3,000 I.U. equine gonadotrophin for 5 days followed by 1,500 I.U. chorionic gonadotrophin on 3 alternate days.

In the male some beneficial results have been obtained by the use of pregnant mares' serum in the treatment of deficient spermatogenesis and by the use of chorionic gonadotrophin in cases of cryptorchidism.

The use of gonadotrophin and pituitary hormones is to some extent limited, since these substances are apt to provoke anti-hormone formation when administered over a long period. Anti-hormones appear irrespective of dosage but are more likely to occur in response to crude pituitary extracts. If used for long periods such preparations, including the relatively purer extracts, may tend to aggravate the deficiency which they are intended to correct. It is therefore advisable to interrupt their administration by rest periods. The length of such intervals should be adjusted according to the duration of the preceding therapy.

#### APPLICATION OF GONADOTROPHIN IN THE FEMALE

The evaluation of the clinical results of endocrine therapy in gynaecology presents some difficulty, for there is no universal agreement upon the meaning of many of the terms applied to menstrual disorders. Even if standard definitions of the present terms could be adopted, there would still remain many non-specific terms which fail to describe the real syndrome. Hence, reports of positive or negative results obtained with this or that preparation in this or that menstrual disorder are frequently meaningless. The following is a list of definitions adopted in this work for the main menstrual disorders classified according to their symptomatology.



## TERMINOLOGY OF MENSTRUAL DISORDERS

*Menstruation*: Bleeding preceded by ovulation, corpus luteum formation and proliferation of the secretory type of endometrium.

*Amenorrhoea*: Absence of bleeding.

(a) Primary: If bleeding has never occurred.

(b) Secondary: Absence of bleeding after occurrence of menses.

*Menorrhagia*: Ovulatory cyclic bleeding, whether more prolonged or more profuse than normal.

*Metrorrhagia*: Non-ovulatory acyclic bleeding, prolonged or profuse.

*Hypermenorrhoea*: Ovulatory cyclic bleeding, excessive but not prolonged.

*Hypomenorrhoea*: Ovulatory cyclic bleeding with scanty flow.

*Oligomenorrhoea*: Ovulatory, cyclic but infrequent bleeding.

*Polymenorrhoea* or *Epimenorrhoea*: Ovulatory, cyclic but over-frequent bleeding.

*Functional Uterine Bleeding*; *Dysfunctional Uterine Bleeding*: Abnormal uterine bleeding, not caused by organic disease, but due to disorders of the endocrine system.

*Abnormal Uterine Bleeding*: Non-ovulatory acyclic uterine bleeding, abnormal in amount and duration.

*Metropathia Haemorrhagica*; *Hyperplastic Bleeding*; *Cystic Glandular Hyperplasia*: Non-ovulatory and acyclic bleeding, excessive or prolonged, from a hyperplastic endometrium. Periods of amenorrhoea, alternating with periods of metrorrhagia, associated with persistent follicles in the ovaries and cystic dysplasia of the endometrium.

*Intermenstrual Bleeding*; *Midmenstrual Bleeding*; *Intracyclic Bleeding*; *Periodic Interval Haemorrhage*; *Midcycle Bleeding*; *Ovulation Bleeding*; *Periodic Staining or Bleeding*: Bleeding occurring approximately midway between the menses.

*Biphasic Bleeding*: Cyclic bleeding at intervals of approximately 2 weeks—an exaggeration of midmenstrual bleeding.

*Vicarious Bleeding*: Cyclic extra-genital bleeding whether or not accompanied by menstrual flow.

*Cyclic non-ovulatory Bleeding*: Cyclic bleeding not preceded by ovulation, corpus luteum formation and progestational endometrium.

*Cyclic Oestrogenic Bleeding*: Cyclic bleeding from an oestrogenic endometrium not necessarily preceded by failure of ovulation.

*Menometrorrhagia*: This term, frequently applied to abnormal uterine bleeding, fails to define the specific nature of the abnormal

bleeding, and is sometimes wrongly used instead of metropathia haemorrhagica.

According to Hamblen [118], the term "Menometrorrhagia", although it is perhaps the one most commonly used, is the least specific and the most frequently abused. If the term is explained according to its three components—meno-metro-rrhagia—it simply means menstrual-uterine-bleeding.

If, however, it is meant to denote a combination of menorrhagia and metrorrhagia, its use becomes even more confusing, since it is contradictory in character, menorrhagia meaning ovulatory, cyclic, prolonged or profuse bleeding, and metrorrhagia meaning non-ovulatory, non-cyclic, prolonged or profuse bleeding.

#### COMPLEXITY OF ENDOCRINE DISORDERS

Menstrual disorders may be caused by numerous non-endocrine factors or by endocrine disturbances which do not respond to hormone therapy. It is obviously of paramount importance to exclude these cases before such treatment is instituted. The aetiology of amenorrhoea may thus be nutritional, nervous, psychological, infectious or neoplastic. Different endocrine changes may produce an identical symptom or even syndrome. On the other hand, various symptoms, either co-existing or alternating at various stages, may be due to a single disorder. The primary cause of amenorrhoea may be either pituitary or ovarian failure. The most marked cases of pituitary amenorrhoea are Simmonds's disease, Fröhlich's syndrome, and the later stages of acromegaly. Amenorrhoea caused by ovarian dysfunction may be due to oestrogen deficiency, or it may occur in the presence of a high oestrogenic output, due to increased follicular growth, persistent cysts or a granulosa-cell tumour. Other ovarian causes of amenorrhoea are a masculinizing arrhenoblastoma or a functioning corpus luteum cyst. In addition to ovarian causes, the disorder of any gland may interfere with regular menses; thus adrenal cortical hyperplasia, thyrotoxicosis and severe hypothyroidism are usually associated with amenorrhoea.

Functional uterine bleeding may be produced by many of the causes responsible also for amenorrhoea. It may be of primary pituitary or ovarian aetiology and may be associated with a low, normal, high, or protracted oestrogenic level. Ovulation may or may not precede the onset of bleeding, which may be cyclic, non-cyclic, prolonged or profuse. Ovarian activity may be decreased or increased, or may present persistent follicular cysts or a granulosa-cell tumour.

Hypothyroidism is frequently accompanied by abnormal uterine bleeding.

A disorder affecting one gland usually involves the whole endocrine system and the diagnosis is often difficult. Treatment should be aimed at correcting the underlying glandular disturbance. This can be achieved either by substitutional or stimulating therapy. The pituitary trophic hormones exert a stimulating effect on the other glands but represent substitution therapy to the pituitary. The effect of this on the pituitary, and whether the glands stimulated to greater

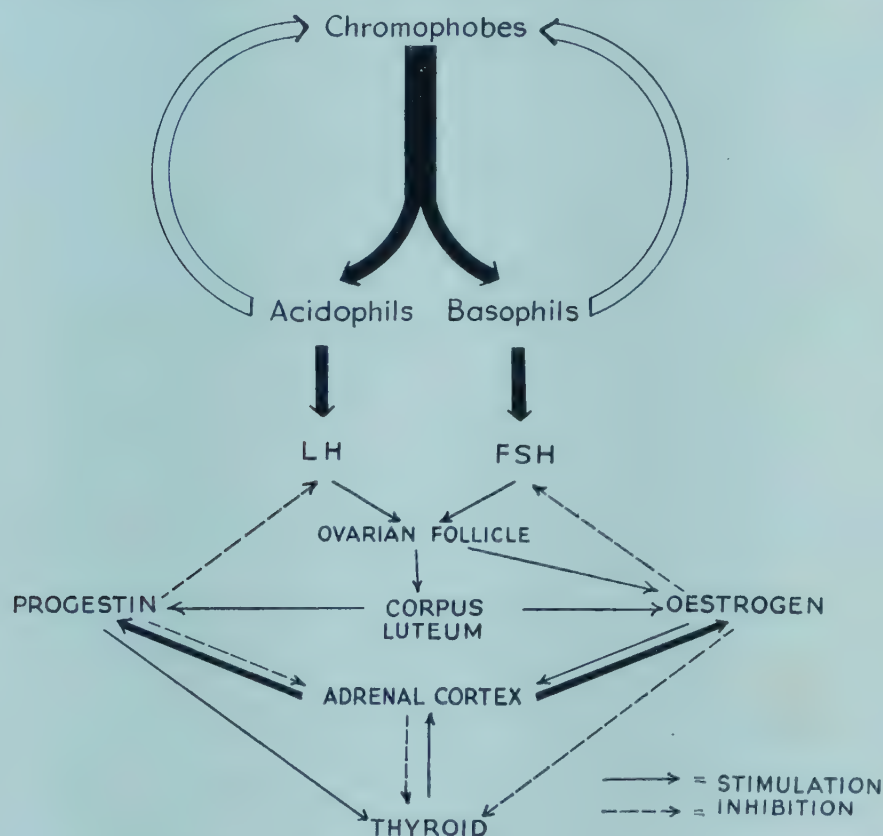


FIG. 4.—THE ANTERIOR PITUITARY-OVARIAN CYCLE IN RELATION TO THE THYROID AND ADRENAL CORTEX

activity may in time increase pituitary function and thus complete a vicious circle, has to await further investigation. Substitution therapy, on the whole, harmful to the gland whose function is replaced by an exogenous hormone. Thus, the administration of oestrogens may inhibit ovarian function, and of androgens depress testicular activity. On the other hand, there is convincing evidence that pituitary function is controlled by the stimulating and inhibitory effects of the other gland hormones. Where decreased glandular function is due to pituitary failure, substitution therapy applied in small doses over a short period of time may stimulate pituitary function, resulting in



increased impulses to the resting gland, and thus re-establish a normal inter-relationship in the endocrine system (see Fig. 4).

#### CLINICAL APPLICATIONS AND APPROXIMATE DOSAGES

Failure to obtain beneficial results with the gonadotrophic hormones may be due to their content of two gonadotrophic factors of unknown ratio. This unknown qualitative relationship of the gonadotrophic hormones does not allow their application in a physiological manner, and the best results so far obtained are those in which treatment was aimed at replacing pituitary function as nearly as possible.

Gonadotrophic preparations are contraindicated in cases of protracted oestrogenic secretion, increased ovarian activity, persistent follicular cysts, or an oestrogen-producing tumour. The following schedules of gonadotrophin therapy may be applied.

#### **Conditions due to Decreased Ovarian Function**

1. Equine gonadotrophin: 400 I.U. daily for 10 days;  
followed by:

Chorionic gonadotrophin: 500 I.U. daily for 10 days.  
Hamblen and others [98-100].

2. Synapoidin: 1 c.c. 3 times weekly for 3 weeks.  
Davis [106].

#### **Oligomenorrhoea**

Equine gonadotrophin: 400 I.U. daily for 10 days;  
followed by:

Chorionic gonadotrophin: 500 I.U. daily for 10 days.  
[98-100.]

(Satisfactory results are reported in more than 50 per cent of cases.)

#### **Polymenorrhoea**

Chorionic gonadotrophin: 500 I.U. daily or every other day starting  
2 weeks after the onset of the preceding flow.

Hoffman [109].

#### **Non-ovulatory Bleeding**

1. Equine gonadotrophin: 400 I.U. daily for 10 days;  
followed by:

Chorionic gonadotrophin: 500 I.U. daily for 10 days.  
Hamblen and others [98-100].

2. Synapoidin: 1 c.c. Five to twenty injections during the first half of the menstrual cycle.

[103.]

If bleeding is prolonged or excessive, haemostatic measures should be applied before institution of treatment.

## CLINICAL APPLICATION OF GONADOTROPHIC PREPARATIONS IN THE MALE

### **Pseudo-hermaphroditism**

Chorionic gonadotrophin: 500 r.u. twice weekly for 5 weeks.

Diaz [114].

### **Eunuchoidism**

Testosterone propionate: 25 mg. 4 times a week for 3 months; followed by:

Chorionic gonadotrophin: 250-500 I.U. 4 times a week for a period of 3-6 months.

Gordon and Fields [115].

### **Cryptorchidism**

1. Chorionic gonadotrophin: 500 I.U. twice weekly for 4-6 weeks. After an interval, a second course of the same type can be repeated.

Gardiner-Hill [90].

2. Gordon and Fields [115] noted a greater effect of chorionic gonadotrophin when its administration was preceded by:

Testosterone propionate: 10-25 mg. twice weekly for 4-8 weeks.

Total dosage: 225-1,470 mg. with an average of 475.5 mg.; and thereafter replaced by:

Chorionic gonadotrophin: 500 I.U. 4 times a week for 1-3 months.

Total dosage: 5,000-95,000 I.U. with an average of 26,595 I.U.

### **Deficient Spermatogenesis**

Equine gonadotrophin: 400-2,000 I.U. intravenously 3 times a week for an average of from 3 to 6 months.

Some beneficial results from this treatment have been reported by Charny [102].

No significant change in seminal values was obtained with any other gonadotrophin treatment either alone or combined or with additional administration of androgens [108].

## BIBLIOGRAPHY

1. SEVERINGHAUS, A. E. *Anat. Rec.* 61 (Suppl.), 61, 1935.
2. TEEL, H. M., and WATKINES, O. *Am. J. Physiol.*, 89, 662, 1929.
3. HOWSE, M. H. *J. Exper. Biol.*, 15, 447, 1938.
4. ZONDEK, B. *Lancet*, 2, 842, 1936.
5. MCEUEN, C. S., SELYE, H., and COLLIP, J. B. *Proc. Soc. Exper. Biol. & Med.*, 63, 390, 1937.
6. THOMPSON, W. O. *J.A.M.A.*, 125, 15, 1944.
7. GREENE, RAYMOND. *Proc. Roy. Soc. London*, 37, 621, 1944.
8. SHELTON, E. K. *Endocrinol.*, 30, 100, 1942.
9. MARINE and ROSEN. *Am. J. Physiol.*, 107, 677, 1934.
10. SAX, M. G. *J. of Physiol. of the U.S.S.R.*, 1, 138, 1940.
11. BOROWSKI, M. L. Laboratory of Histo-Pathology of the Nervous System, Moscow, 1944.
12. FLEISCHMAN, W. *J. Clin. Endocrinol.*, 1, 98, 1941.
13. THOMPSON, W. O. and P. K., and TAYLOR, S. G. *Endocrinol.*, 48, 633, 1940.
14. LERMAN, J., and STEBBINS, H. D. *J.A.M.A.*, 119, 391, 1942.
15. WHITEHEAD, R. *Brit. J. Exp. Path.*, 13, 200, 1932.
16. FAHR, T. H. *Verhandl. D. Path. Ges.*, 15, 234, 1912.
17. TEPPERMAN, J., and ENGLES, F. G. Mayo Foundation, 1942.
18. BOURNE, G., and ZUCKERMAN, S. *J. Endocrinol.*, 2, 283, 1940.
19. HALL, K., and KORENCHEVSKY, K. *J. Physiol.*, 91, 365, 1938.
20. SCHMIDT, I. G., and SCHMIDT, L. H. *Endocrinol.*, 23, 559, 1938.  
NELSON, D. *Fed. Proc.*, 1, 62, 1942.
21. ROSEN, S. H., and MARINE, D. *Proc. Soc. Exper. Biol. & Med.*, 41, 647, 1939.
22. LOWENSTEIN, B. E., and ZWEMER, R. L. *Endocrinol.*, 30, 135, 1942.
23. GOLDZIEHER, M. A. "Adrenal Glands in Health and Disease", F. A. Davis Co., Philadelphia, 1944.
24. SEVERINGHAUS, A. E. *Physiol. Rev.*, 17, 566, 1937.
25. NOBLE, R. L., and COLLIP, J. B. *Endocrinol.*, 29, 934, 1941.
26. EVANS, H. M., SIMPSON, M. E., and LI, CHOH H. *Endocrinol.*, 33, 237, 1943.
27. BECKS, H., SIMPSON, M. E., LI, CHOH H., and EVANS, H. M. *Endocrinol.*, 34, 305, 1944.
28. MARKS, W., SIMPSON, M. E., LI, CHOH H., and EVANS, H. M. *Endocrinol.*, 33, 102, 1943.



29. BECKS, H., SIMPSON, M. E., MARKS, W., LI, CHOH H., and EVANS, H. M. *Endocrinol.*, **34**, 311, 1944.
30. SIMPSON, M. E., MARKS, W., BECKS, H., and EVANS, M. R. *Endocrinol.*, **35**, 234, 1944.
31. TORDA, C., and WOLFF, H. G. *Proc. Soc. Exper. Biol. & Med.*, **57**, 69, 1944.
32. *Ibid.*, **57**, 137, 1944.
33. HEMPHILL, R. E., and REISS, M. *J. Ment. Sc.*, **86**, 1065, 1940.
34. *Ibid.*, **88**, 559, 1942.
35. HEMPHILL, R. E. *J. Ment. Sc.*, **88**, 285, 1942.
36. HEMPHILL, R. E., and REISS, M. *Brit. Med. J.* 5362, 211, Aug. 1944.
37. SCHOOLEY, J. P., and RIDDLE, O. *Am. J. Anat.*, **62**, 313, 1933.
38. RIDDLE, O., and BATES, R. W. *Endocrinol.*, **17**, 689, 1933.
39. RIDDLE, O., and BATES, R. W. *Proc. Soc. Exper. Biol. & Med.*, **32**, 730, 1935.
40. BATES, R. W., LAHR, E. L., and RIDDLE, O. *Am. J. Physiol.*, **111**, 361, 1935.
41. SCHOOLEY, J. P., RIDDLE, O., and BATES, R. W. *Anat. Rec.*, **72**, 90, 1938.
42. REECE, R. P., and TURNER, C. W. *Proc. Soc. Exper. Biol. & Med.*, **36**, 283, 1937.
43. GRAHAM, W. R. JNR. *J. Nutr.*, **7**, 407, 1934.
44. RALSTON, N. P., COWSERT, W. C., RAGSDALE, A. C., HERMAN, H. A., and TURNER, C. W. *Missouri Agr. Exper. Sta. Res. Bull. No. 317*, 1940.
45. KENNY, M., KING, E., EVERS, N., and HUNAN, W. J. *Lancet*, **2**, 828, 1939.
46. WINSON, S. G. *Am. J. Obst. & Gynec.*, **46**, 545, 1943.
47. DOUGHERTY, T. F., and WHITE, A. *Endocrinol.*, **39**, 370, 1946.
48. FRANK, R. T. "Glandular Physiology and Therapy", American Medical Association, Chicago, 1935.
49. DRIPS, D. J., and OSTERBERG, A. E. *Endocrinol.*, **23**, 703, 1938.
50. D'AMOUR, F. E. *J. Clin. Endocrinol.*, **3**, 41, 1943.
51. HAMBLIN, E. C. "Endocrinology of Women", p. 149, Charles C. Thomas, Springfield, Illinois, 1945.
52. FEVOLD, H. L. *Endocrinol.*, **24**, 435, 1939; *J. Biol. Chem.*, **128**, 83, 1939.
53. FEVOLD, H. L., LEE, M., HISAW, F. L., and COHN, E. J. *Endocrinol.*, **26**, 999, 1940; *ibid.*, **27**, 781, 1940.
54. SHEDLOVSKY, T., ROTHEN, A., GREEP, R. O., VAN DYKE, H. B., and CHOW, B. F. *Science*, **92**, 178, 1940.
55. GREEP, R. O., VAN DYKE, H. B., and CHOW, B. F. *J. Biol. Chem.*, **133**, 289, 1940; *Endocrinol.*, **30**, 635, 1942.
56. EVANS, H. M., SIMPSON, M. E., TOLKSDORF, S., and JENSEN, H. *Endocrinol.*, **25**, 529, 1939.
57. TOLKSDORF, S., and JENSEN, H. *Proc. Soc. Exper. Biol. & Med.*, **42**, 466, 1939.
58. JENSEN, H., TOLKSDORF, S., and BAUMAN, F. B. *J. Biol. Chem.*, **135**, 791, 1940.

59. LI, CHOH H., SIMPSON, M. E., and EVANS, H. M. *Endocrinol.*, **27**, 803, 1940.
60. FEVOLD, H. L. *Endocrinol.*, **28**, 33, 1941.
61. CASIDA, L. E. *Endocrinol.*, **18**, 714, 1934.
62. FOSTER, M. A., and HISAW, F. L. *Anat. Rec.*, **62**, 75, 1935.
63. SMITH, O. W., SMITH, G. V. S., and SCHILLER, S. *Am. J. Obst. & Gynec.*, **45**, 15, 1943.
64. BROOKS, C. "The Hypothalamus and Central Levels of the Autonomic Function", Williams & Wilkins Co., Baltimore, 1940.
65. WESTMAN, A., JACOBSON, D., and HILLARP, N. A. *Monatschrift f. Geburt. u. Gynäkologie Basel*, **116**, 225, 1943.
66. PFEIFFER, C. H., *Ann. Rev. Physiol.*, **5**, 413, 1943.
67. WOLFE, T. M., and BROWN, A. D. *Endocrinol.*, **31**, 467, 1942.
68. KLAFTEN, E. *Ztschr. f. Geburtsh u. Gynäk.*, **115**, 64, 1937.
69. ABARBANEL, A. R. Annual Meeting of the Society for the Study of Internal Secretions, Chicago, 1944.
70. MAKEPEACE, A. W., WEINSTEIN, G. Z., and FRIEDMAN, M. H. *Am. J. Physiol.*, **119**, 812, 1937.
71. GREEP, R. O. *Endocrinol.*, **23**, 154, 1938.
72. GAARENSTROOM, J. J., and DE JONGH, S. E. *Acta. Brev. Neerland*, **10**, 202, 1940.
73. PENCHARZ, R. I. *Science*, **91**, 554, 1940.
74. SIMPSON, M. E., EVANS, H. M., FRANCKEL-CONRAD, H. L., and LI, CHOH H. *Endocrinol.*, **28**, 37, 1941.
75. BROWN, W. E., BRADBURY, J. T., and METZGER, I. *Am. J. Obst. & Gynec.*, **41**, 582, 1941.
76. CATCHPOLE, H. R., and LYONS, W. R. *Am. J. Anat.*, **55**, 167, 1934.
77. EVANS, H. M., KORPI, K., SIMPSON, M. E., PENCHARZ, R. J. *Pub. Anat.*, **1**, 275, 1936.
78. LIU, S. H., and NOBLE, R. S. *J. Endocrinol.*, **1**, 7, 1939.
79. EVANS, H. M., MEYER, K., and SIMPSON, M. E. *Proc. Soc. Exper. Biol. & Med.*, **28**, 845, 1931.
80. FEVOLD, H. D., HISAW, F. L., HELLBAUM, A., and HERTZ, R. *Proc. Soc. Exper. Biol. & Med.*, **30**, 914, 1933.
81. FEVOLD, H. D., and HISAW, F. L. *Am. J. Physiol.*, **190**, 655, 1934.
82. LEIN, A. *Proc. Soc. Exper. Biol. & Med.*, **36**, 609, 1937.
83. DAVIS, M. E., and HELLBAUM, A. A. *J. Clin. Endocrinol.*, **4**, 400, 1944.
84. RYDBERG, E., and PEDERSEN-BJERGAARD, J. *J.A.M.A.*, **121**, 117, 1943.
85. HAMBLIN, E. C., and DAVIS, C. D. *Am. J. Obst. & Gynec.*, **50**, 137, 1945.
86. HAMBLIN, E. C. "Endocrine Gynaecology", Baillière, Tindall & Cox, London, 1939.
87. GEIST, S. H. *Am. J. Onst. & Gynec.*, **26**, 588, 1933.
88. ELLISON, E. T., and BURCH, G. D. *Endocrinol.*, **20**, 746, 1936.
89. GORDON, M. R., and FIELDS, E. M. *J. Clin. Endocrinol.*, **2**, 715, 1942.
90. GARDINER-HILL, H. *Practitioner*, **152**, 94, 1944.
91. DAVIS, M. E., and KOFF, A. K. *Am. J. Obst. & Gynec.*, **36**, 183, 1938.

92. SIEGLER, S. L., and FEIN, M. J. *Am. J. Obst. & Gynec.*, **28**, 1021, 1939.
93. GRIFFITHS, L. S., and MCBRIDE, W. P. L. *Am. J. Obst. & Gynec.*, **43**, 1012, 1942.
94. IRVING, H. W., SEARS, C., and ROCK, J. *Am. J. Obst. & Gynec.*, **40**, 695, 1940.
95. BREWER, J. I., JONES, H. O., and SKILES, J. H., JR. *J.A.M.A.*, **118**, 278, 1942.
96. GEIST, S. H., GAINES, J. A., and SALMON, U. J. *Am. J. Obst. & Gynec.*, **42**, 619, 1941.
97. BAKER, B. L., and EVERETT, N. B. *Endocrinol.*, **34**, 254, 1944.
98. HAMBLIN, E. C., CUYLER, W. K., WILSON, J. A., and PULLEN, R. L. *J. Clin. Endocrinol.*, **1**, 749, 1941.
99. VOGT, W. H., and SEXTON, D. L. *Am. J. Obst. & Gynec.*, **42**, 81, 1941.
100. HAMBLIN, E. C. *Am. J. Obst. & Gynec.*, **41**, 495, 1941.
101. DAVIS, C. D., PULLEN, R. L., MADDEN, J. H. M., and HAMBLIN, E. C. *J. Clin. Endocrinol.*, **3**, 268, 1943.
102. CHARNY, C. W. *Am. J. Med. Sci.*, **207**, 519, 1944.
103. MAZER, C., and RAVETZ, E. *Am. J. Obst. & Gynec.*, **41**, 474, 1941.
104. MAZER, C., and ISRAEL, S. L. "Diagnosis and Treatment of Menstrual Disorders and Sterility", Heinemann, London, 1941.
105. GEIST, S. H., GAINES, G. A., and SALMON, U. J. *Am. J. Obst. & Gynec.*, **42**, 619, 1941.
106. DAVIS, A. J. *J. Obst. & Gynec. Brit. Emp.*, **51**, 401, 1944.
107. HOFFMAN, J. "Female Endocrinology", p. 349, W. B. Saunders Co., Philadelphia, 1944.
108. DAVIS, C. D., MADDEN, J. H. M., and HAMBLIN, E. C. *J. Clin. Endocrinol.*, **3**, 357, 1943.
109. HOFFMAN, J. "Female Endocrinology", W. B. Saunders Co., Philadelphia, 1944.
110. HILLS, A. G., FORSHAM, P. H., and FINCH, C. A. Twenty-Ninth Ann. Meet. of Assn. for Study of Internal Secretions, 1947.
111. DEANE, H. W., and BERGNER, G. E. Twenty-Ninth Ann. Meet. of Assn. for Study of Internal Secretions, 1947.
112. GORDAN, G. S., LI, CHOH H., and BENNETT, L. L. *Proc. Soc. Exper. Biol. & Med.*, **62**, 103, 1946.
113. YOFFEY, J. M., REISS, M., and BAXTER, J. S. *Nature*, **157**, 368, 1946.
114. DIAZ, J. T. *Am. J. Dis. Child.*, **65**, 67, 1943.
115. GORDON, M. B., and FIELDS, E. M. *J. Clin. Endocrinol.*, **3**, 589, 1943.
116. WILLIAMSON, M. B. *Proc. Soc. Exper. Biol. & Med.*, **63**, 191, 1946.
117. LEATHEM, J. H., and RAKOFF, A. E. Twenty-Ninth Ann. Meet. of Assn. for Study of Internal Secretions, 1947.
118. HAMBLIN, E. C. "Endocrinology of Women", Chas. C. Thomas, Springfield, Illinois, 1945.
119. CAMPBELL, E., and SEVERINGHAUS, E. L. *Am. J. Obst. & Gynec.*, **37**, 913, 1939.



120. SEVERINGHAUS, E. L. *West. J. Surg.*, **51**, 153, 1943.
121. SEVERINGHAUS, E. L. *Bull. New York Acad. Med.*, **16**, 53, 1940.
122. SEVERINGHAUS, E. L. "Endocrine Therapy in General Practice", *Year Book*, Publishers Inc., Chicago, 1938.
123. KNAUS, H. *Zentralbl. f. Gynäk.*, **59**, 2642, 1935.
124. CHASSAR MOIR. *J. Obst. & Gynec. Brit. Emp.*, **3**, 181, 1944.
125. METZ, H. M., and LACKEY, R. W. *South. Med. J.*, **36**, 747, 1943.
126. GREENE, J. A., and JANUARY, L. E. *Proc. Soc. Exper. Biol. & Med.*, **44**, 217, 1940.
127. STEPHENS, D. J. *Proc. Soc. Exper. Biol. & Med.*, **44**, 240, 1940.
128. BROWN, W. E., and BRADBURY, J. T. *Am. J. Obst. & Gynec.*, **53**, 748, 1947.
129. HISAW, F. L. Am. Soc. for the Study of Sterility, Third Annual Convention, 1947.
130. HELLBAUM, A. A., and GREEP, R. O. *Proc. Soc. Exper. Biol. & Med.*, **63**, 53, 1946.
131. MEYER, R. K., BIDDULPH, C., and FINERTY, J. C. *Endocrinol.*, **39**, 23, 1946.
132. GOLDZIEHER, M. A. *J. Clin. Endocrinol.*, **5**, 132, 1945.
133. KUPPERMAN, H. S., FRIED, P., and HAIR, L. Q. *Am. J. Obst. & Gynec.*, **48**, 228, 1944.
134. HALL, G. J. *J. Clin. Endocrinol.*, **2**, 296, 1942.
135. FOLLEY, S. J., and MALPRESS, F. H. *J. Endocrinol.*, **4**, 1, 1944.
136. SCHOOLEY, J. P., and RIDDLE, O. *Am. J. Anat.*, **62**, 313, 1938.
137. SMELSER, G. K. *Endocrinol.*, **34**, 39, 1944.
138. FLUHMAN, C. F. *Endocrinol.*, **17**, 550, 1933; *Am. J. Obst. & Gynec.*, **28**, 668, 1934.
139. WILLIAMS, P. C. *Nature*, **145**, 388, 1940.
140. FEVOLD, H. L. "Sex and Internal Secretions", p. 966, The Williams & Wilkins Co., Baltimore, 1939.
141. MEITES, J., and TURNER, C. W. *Proc. Soc. Exper. Biol. & Med.*, **49**, 190, 1942.
142. MEITES, J., and TURNER, C. W. *Endocrinol.*, **30**, 711, 1942.
143. ESKIN, I. A. *Bull. Exper. Biol. & Med. U.S.S.R.*, **7-8**, 68, 1942.
144. BROWN, W. E., and BRADBURY, J. T. Am. Soc. for the Study of Sterility, Third Annual Convention, 1947.
145. AZIMOV, G. L., and ALTMAN, A. D. *Compt. Rend. de l'Acad. des Sci. de l'U.S.S.R.*, **20**, 621, 1938.
146. FRIEDMAN, M. H., and HALL, S. Twenty-Fifth Ann. Meet. and Scientific Session Assn. for Study of Internal Secretions, p. 10, 1941.
147. GOLDBERG, M. B., and LISSER, H. *J. Clin. Endocrinol.*, **2**, 477, 1942.
148. HUTTON, E. L., and REISS, M. *J. Ment. Sc.*, **88**, 550, 1944.
149. SCHRIRE, I., and SHARPEY-SCHAFFER, E. P. *Clin. Sc.*, **3**, 413, 1938.
150. SCHRIRE, I. *Quart. J. of Med.*, **6**, 17, 1933.
151. SCHRIRE, I., and SHARPEY-SCHAFFER, E. P. *Clin. Sc.*, **3**, 369, 1938.
152. SHARPEY-SCHAFFER, E. P., and SCHRIRE, I. *Clin. Sc.*, **4**, 185, 1939.

- I53. SHARPEY-SCHAFFER, E. P., and SCHRIRE, I. *Quart. J. of Med.*, **8**, 195, 1939.
- I54. SCHRIRE, I., and ZWARENSTEIN, H. *Biochem. J.*, **27**, 1337, 1933.
- I55. *Ibid.*, **28**, 356, 1934.
- I56. *Ibid.*, **26**, 118, 1932.
- I57. *Ibid.*, **26**, 1886, 1932.
- I58. CUMINGS, J. N. *Brain*, **67**, 265, 1944.
- I59. LI, CHOH H., and EVANS, H. M. *Science*, **99**, 183, 1944.
- I60. EVANS, H. M., SIMPSON, M. E., and LI, CHOH H. Twenty-Eighth Ann. Meet. of Assn. for Study of Internal Secretions, 1946.
- I61. FINKLER, R. S., FURST, N. G., and COHN, G. M. *J. Clin. Endocrinol.*, **2**, 603, 1942.
- I62. GORDON, M. B., and FIELD, E. M. *J. Clin. Endocrinol.*, **2**, 715, 1942.
- I63. KENYON, A. T., KNOWLTON, K., and SANDIFORD, S. *Ann. Int. Med.*, **20**, 632, 1944.
- I64. PURVES, H. D., and GRIESBACH, W. E. *Endocrinol.*, **39**, 274, 1946.
- I65. SAYERS, G., and SAYERS, M. A., *Endocrinol.*, **40**, 265, 1947.
- I66. MUNSON, P. L., and KOCH, F. C. Twenty-Eighth Ann. Meet. of Assn. for Study of Internal Secretions, 1946.
- I67. BENNETT, L. L., and LI, CHOH H. Twenty-Eighth Ann. Meet. of Assn. for Study of Internal Secretions, 1946.
- I68. BENNETT, L. L., APPLGARTH, A. P., and LI, CHOH H. *Proc. Soc. Exper. Biol. & Med.*, **65**, 265, 1947.
- I69. REECE, R. P., and TURNER, C. W. *Mo. Agr. Exper. Sta. Res. Bull. No.* 266, 1937.
- I70. LEWIS, A. A., and TURNER, C. W. *Proc. Soc. Exper. Biol. & Med.*, **48**, 439, 1941.
- I71. MEITES, J., TRENTIN, J. J., and TURNER, C. W. *Endocrinol.*, **31**, 607, 1942.
- I72. WAINMAN, P., REESE, J. D., and KONEFF, A. A. *Endocrinol.*, **31**, 303, 1942.
- I73. MEITES, J., and TURNER, C. W. *Proc. Soc. Exper. Biol. & Med.*, **49**, 190, 1942.
- I74. FOSTER, C. L. *J. Endocrinol.*, **3**, 79, 1942.
- I75. BAKER, B. L., and EVERETT, N. B. *Endocrinol.*, **34**, 254, 1944.
- I76. ZECKWER, I. T. *Science*, **100**, 123, 1944.
- I77. WOLFE, J. M. *Endocrinol.*, **29**, 969, 1941.

## POSTERIOR PITUITARY HORMONES

It was formerly believed that the posterior lobe hormones, pressor, oxytocic, anti-diuretic and melanophore-dispersing principles were elaborated in the pars intermedia and stored in the pars nervosa. Convincing evidence, however, supplied in the last few years has shown that the pressor, oxytocic and anti-diuretic factors are produced in the pars nervosa, and the melanophore-dispersing principle in the pars intermedia. The primary structural elements of the pars nervosa are pituicytes and unmyelinated nervous fibres. Changes in the pituicytes have been correlated with secretory activity.

Two highly purified fractions, pitressin and pitocin, have been isolated from pituitary extracts—pitressin possessing pressor and anti-diuretic properties and pitocin possessing oxytocic properties. Pituitrin is the extract containing both active principles.

## PITUITRIN (PITRESSIN AND PITOCIN)

## PHYSIOLOGY

Both pitressin and pitocin cause hyperglycaemia and act as antagonists to insulin, presumably owing to their direct action on the liver cells. Administration of pituitrin produces increased formation of lactic acid, considerable lowering of the carbon-dioxide-combining power of the blood, increase in organic phosphates, and decreased utilization of oxygen by the tissues.

## PITRESSIN

## PRESSOR EFFECT

Injection of pitressin causes coronary constriction. In experimental animals the blood-pressure is markedly raised after injection of a solution of pituitrin or pitressin. Respiratory changes caused by pitressin are secondary to the circulatory effects.

## ANTI-DIURETIC EFFECT

Injections of pituitrin or pitressin cause a markedly anti-diuretic response in patients with diabetes insipidus, or in normal subjects after ingestion of water, the effect lasting a few hours. Destruction of the pars nervosa will cause diabetes insipidus only in the presence of an intact anterior lobe, and it has been suggested that the anterior lobe produces a diuretic substance which, under normal conditions, is antagonized by the anti-diuretic factor of the posterior lobe.



## RELATION TO OTHER FACTORS

*(See Fig. 1)***Adrenal Cortex**

The relation of the adrenal cortex to the posterior lobe of the pituitary is one of synergism for the maintenance of normal water balance. If, however, the balance between the posterior lobe of the pituitary and the adrenal cortex is disturbed by failure of one of the glands, an antagonism is created with consequent hyperfunction of the other gland in the sense that the posterior lobe exerts an anti-diuretic and the adrenal gland a diuretic effect.

Polyuria, produced by hypophysectomy, is checked by adrenalectomy [1]. Adrenalectomy, on the other hand, is soon followed by an increase in the amount of anti-diuretic substance of pituitary origin present in the urine (see Chapter V).

**Desoxycortone Acetate**

Injection of desoxycortone acetate increases water intake and output, and reduces sodium and chloride excretion. Administration of posterior lobe extract restores normal water metabolism [2].

**Medulla**

Adrenalin is synergic to pitressin, since the latter sensitizes the tissues to the effects of adrenalin. Both substances given separately have hyperglycaemic effects. Combined administration, however, inhibits the increase in sugar.

An antagonism exists between adrenalin and pitressin in their action on the uterine muscle and on renal function, since adrenalin produces diuresis and its inhibiting effect upon the myometrium is reversed by posterior lobe extracts.

**Nicotine**

Nicotine exerts an anti-diuretic effect in the rat and human, and it is suggested that the anti-diuretic action of smoking is presumably due to the nicotine absorbed from the cigarette smoke stimulating the hypothalamic supra-optic nucleus which causes a discharge of the posterior pituitary lobe hormone [3].

**Pregnancy Hormones**

It has frequently been suggested that concentrates of urine from pregnant women with toxæmia have an anti-diuretic effect due to an

increased function of the posterior lobe [4]. Ham [5], however, demonstrated that the anti-diuretic factor in the urine of both pregnant rats and human beings is not identical with that of the posterior lobe, but is of placental origin.

### **Gastro-intestinal Tract**

Pitressin stimulates intestinal peristalsis causing an urgent desire to empty the bowel, whereas pitocin exerts the opposite effect. Administration of pitressin causes marked inhibition of secretion of hydrochloric acid.

### **PITOCIN**

There is some controversy as to whether or not the oxytocic factor is essential for parturition. No convincing proof has been supplied one way or the other.

The reaction of the uterus, non-pregnant or pregnant, to pitocin is, to some degree, controlled by the ovarian, placental and anterior pituitary hormones. During the early stage of pregnancy the human uterus is refractory to pitocin but responds to small doses of pitressin. The reactivity to pitocin returns at a later stage of pregnancy and is greatly increased at term. Oestrogen renders the gravid uterus highly reactive to pitocin. It was recently shown, however, that blood from human beings from the fifth month of pregnancy to at least 7 days postpartum rapidly inactivates pitocin and pitressin as tested by their oxytocic action on the uterus. This fact is reported as evidence that the posterior pituitary is not the causative agent of parturition in human beings [6].

The non-gravid uterus responds to pituitrin more actively than to pitocin. The uterine response to pituitrin was believed by Knaus [7] to be high during the first half of the menstrual cycle only, and lost within 48 hours of follicular rupture. Chassar Moir [8], however, investigated the response of the human uterus to posterior lobe pituitary gland extract by the intra-uterine bag method, and contrary to Knaus's observations, found that the non-pregnant uterus responds to posterior lobe pituitary extract at every phase of the menstrual cycle. In the first half of the menstrual cycle the uterine contractions are irregular, small in magnitude, and separated by intervals of 30-60 seconds. At about mid-cycle there is a gradual change, the small contractions disappearing and being replaced by much larger and more prolonged contractions which come at intervals of 2-3 minutes. As the cycle advances, they become stronger, more regular and

usually more frequent, eventually merging with the contractions of menstruation.

It seems that oestrogen sensitizes the uterus to the vasoconstrictor principle of the posterior lobe, whereas progestogen inhibits its release. After the regression of the corpus luteum and the cessation of progestogen secretion, the inhibitory effect on the posterior lobe is removed, and the release of its vasoconstrictor principle acting on the oestrogen-sensitized mucosa causes pre-menstrual vasoconstriction of the uterine vessels.

### PARS INTERMEDIA

The hormones of the pars intermedia are intermedin and the melanophore-expanding principle. Chromatophore-expanding substances have been obtained from human urine, particularly during pregnancy.

Extracts of posterior lobes exert a chromatophore-expanding action. The melanophore-expanding fraction is a powerful anti-diuretic which has been successfully used in a limited number of cases of diabetes insipidus.

There are no commercial preparations available of the pars intermedia.

### STANDARDIZATION

#### **Pituitrin**

An international standard, 0.5 mg. of dried powdered posterior pituitary gland (derived from 3.5 mg. of the fresh glandular material), has been adopted.

#### **Pitressin**

For the determination of its pressor activity, the decerebrated cat or decapitated rat is used. The anti-diuretic potency is established by the amount of extract required to delay the excretion of administered water in rats or mice.

#### **Pitocin**

Its oxytocic action is determined on the isolated uterus of the virgin guinea pig. The oxytocic effect of the unknown extract is compared with the official standard preparation.



### Pars Intermedia

To establish its melanophore-expanding action, the normal frog is used, and for its erythrosin-expanding activity the *Phoxinus laevis* is used.

#### PREPARATIONS FOR CLINICAL USE

Pitocin (oxytocic):

Ampoules of 0.5 c.c. and 1 c.c. (1 c.c. contains 10 I.U.).

Pitressin (pressor and anti-diuretic):

Ampoules of 0.5 c.c. and 1 c.c. (1 c.c. contains 10 or 20 pressor units).

Pitressin (tannate in oil):

1 c.c. contains 5 pressor units.

Pituitrin (pressor, oxytocic and anti-diuretic):

Ampoules (surgical) of 0.5 c.c. and 1 c.c. (1 c.c. contains 20 I.U.).

Ampoules (obstetrical) of 0.5 c.c. and 1 c.c. (1 c.c. contains 10 I.U.).

Posterior Pituitary Desiccated Powder. Vials of 1 dram for intranasal insufflation.

#### ADMINISTRATION

Posterior pituitary solutions are injected intramuscularly. Dried powder of the posterior lobe is administered by insufflation high in the nostril.

#### CLINICAL APPLICATION AND APPROXIMATE DOSAGES

##### Obstetrical Conditions

Induction of Labour.

Stimulation of Uterine Contractions.

Control of Post-partum Haemorrhage.

Uterine Inertia.

Pitocin or Pituitrin: 0.5–1 c.c.

An increased effect of pitocin or pituitrin is obtained by the preceding administration of oestrogens (see Chapter VII).

Pituitrin or pitocin should only be used as an oxytocic after the cervix has dilated, and where there is no evidence of dystocia. The strength of the contractions may otherwise lead to rupture of the uterus, foetal asphyxia and laceration of the cervix.

### Diabetes Insipidus

Pitressin: 0.5–1 c.c. intramuscularly every 4 hours,  
or: Posterior Pituitary Powder by nasal insufflation.

### Peptic Ulcer

$\frac{2}{3}$  grain posterior pituitary powder by nasal insufflation 4 times a day;  
30 minutes after each meal and at bedtime.

Metz and Lackey [9].

Metz and Lackey also recommend the use of pitressin in the following conditions:

### Post-operative Abdominal Distension and Paralytic Ileus

Pitressin: 0.5–1 c.c. intramuscularly every 4 hours.

### Pyelitis

Pitressin: 5–20 minims intramuscularly every 4 hours.

## BIBLIOGRAPHY

1. COREY, E. L., SILVETTE, H., and BRITTON, S. W. *Am. J. Physiol.*, **125**, 644, 1939.
2. MULINOS, M. G., SPINGARN, C. L., and LOYGKIN, L. E. *Am. J. Physiol.*, **135**, 102, 1942.
3. CLIFFORD WILSON, POLLOCK, M. R., and HARRIS, A. D. *Brit. Med. J.*, 4394, 399, 1945.
4. TEEL, H. N., and REID, D. E. *Endocrinol.*, **24**, 297, 1939.
5. HAM, G. C. *J. Clin. Invest.*, **20**, 439, 1941.
6. WOODBURY, R. A., AHLQUIST, R. P., ABREAN, B., TORPIN, R., and WATSON, W. G. *J. Pharmacol. & Exper. Therap.*, **86**, 359, 1946.
7. KNAUS, H. *Zentralbl. f. Gynäk.*, **59**, 2642, 1935.
8. CHASSAR MOIR. *J. Obst. & Gynec. Brit. Emp.*, **3**, 181, 1944.
9. METZ, H. M., and LACKEY, R. W. *South. Med. J.*, **36**, 747, 1943.

## THYROID HORMONE

THE chief function of the thyroid gland is the elaboration and storage of the hormone thyroglobulin, which contains the amino-acid thyroxin.

The gland is composed of a number of follicles or acini, each one of which is a secretory unit. The follicular walls consist of epithelium, the height of which changes according to the activity of the gland, becoming flat when thyroid function is diminished. The storage of the elaborated hormone takes place in the lumen of the follicles. The thyroid is equipped with a rich capillary network, through which the blood volume of normal man passes about once an hour. There is some controversy as to whether the rich network of the autonomic nervous fibres, sympathetic from the cervical ganglions and parasympathetic from the vagus, exerts on the thyroid a secretory or a vasomotor action.

Of the total iodine in the normal thyroid gland, about 30 per cent is in the form of thyroxin and about 70 per cent in the form of diiodotyrosine.

For clinical use, desiccated thyroid, thyroxin and thyroglobulin are available.

### PHYSIOLOGY

The normal activity of the thyroid gland depends on (a) the release of thyrotrophic hormone from the anterior pituitary, (b) the availability of adequate amounts of iodine, (c) the inter-relationship of the thyroid gland with the other endocrine glands, particularly the pituitary, adrenal cortex and the gonads, (d) adequate nutrition, and (e) suitable climatic conditions.

#### Temperature

Low temperature increases and high temperature lowers the function of the thyroid gland. In animals living in a cold climate, the thyroid gland has a tall epithelium. The colloid in the follicles becomes rich. On the other hand, at high temperatures, the follicles of the gland are filled with a dense homogeneous colloid, and the intensity of hormone secretion is very much decreased [1, 2].

#### Basal Metabolism

Thyroid deficiency causes a reduction in the metabolic rate. Administration of thyroid results in an increase of metabolic activity with



Increased oxygen consumption and carbon dioxide elimination. One grain of desiccated thyroid increases the B.M.R. of a normal adult by about 2.8 per cent, although a person suffering from myxoedema requires only about 2 grains of desiccated thyroid per day to maintain a normal B.M.R.

Ingestion of a protein meal increases the B.M.R. by about 16.3 per cent (specific dynamic action of proteins, S.D.A.) [3], which presumably is due to the direct action of proteins on the pituitary gland.

The B.M.R. is influenced by age, being highest in childhood and lowest in old age. Women show an average of about 7.1–7.2 per cent lower B.M.R. than men of the same age. There is an increase of metabolism during the pre-menstrual week averaging from 2.7 per cent to 3.7 per cent and a slight fall during menstruation [4, 5]. An increase in the basal metabolic rate occurs during pregnancy.

### **Carbohydrate Metabolism**

Excessive thyroid feeding depletes the liver and muscle glycogen, exerting its action apparently by increasing the effect of adrenalin. Sugar tolerance is often low in Graves's disease and high in myxoedema.

### **Protein Metabolism**

The thyroid hormone causes breakdown of exogenous and endogenous protein, raising the level of urea and creatine.

### **Fat Metabolism**

Patients suffering from hypothyroidism tend to accumulate fat, whereas those suffering from Graves's disease tend to lose it progressively. It is suggested that hyperthyroidism or thyroid feeding causes fat consumption by stimulating muscular activity and by its diuretic effect.

### **Calcium Metabolism**

Hypothyroidism is associated with increased retention of calcium and phosphorus, whereas increased excretion occurs in hyperthyroidism or thyroid feeding and may lead to osteoporosis. It is not clear whether the thyroid hormone influences calcium metabolism directly or through the parathyroids.

### **Water Metabolism**

Hyperthyroidism is frequently associated with polyuria, and hypothyroidism with oliguria. Hypothyroidism is accompanied by tissue

water retention and low plasma volume. Thyroid feeding seems to increase capillary permeability, allowing the water to pass from the tissues into the circulation. The thyroid is probably antagonistic to the anti-diuretic factor of the posterior lobe, for thyroidectomy improves diabetes insipidus.

### **Renal Function**

Renal function is impaired in pituitary hypothyroidism and myxoedema [6, 7].

### **Circulatory System**

Hyperthyroidism is characterized by an increased cardiac output with an increase in the blood flow through the periphery owing to peripheral vaso-dilatation. In hypothyroidism the reverse is observed, i.e. diminished cardiac tone with subsequent enlargement, slow pulse rate and oedema.

### **Nervous System**

Excess of thyroid hormone produces emotional instability, increased nervous irritability and, sometimes, disturbances in cerebation. Hypothyroidism is connected with a low emotional level, sluggish reaction and slow cerebation (see Chapter XII).

On the vegetative nervous system, hyperthyroidism acts by increasing vasomotor activity, peristalsis and the activity of the sweat glands. The reverse takes place in hypothyroidism.

### **Muscular System**

Excess of thyroid hormone induces muscular changes from mild myasthenia to advanced muscular atrophy. Muscular hypotonus is observed in hypothyroidism.

## **RELATION OF THYROID GLAND TO OTHER ENDOCRINE GLANDS (See Figs. 1 and 4)**

### **Hypophysis**

#### *Effects of Thyroidectomy*

Total thyroidectomy causes pituitary hypertrophy [8], marked decrease or complete disappearance of acidophils, increased thyrotrophic hormone, decreased growth hormone and improvement of diabetes insipidus.

Clinically abrupt discontinuation of thyroid medication may be

followed by thyroxicosis with exophthalmos [56, 60]. This is probably due to an increased release of thyrotrophic hormone from the basophilic cells following inhibition of their function by thyroid administration.

### *Excess of Thyroid Hormone*

Thyroxin has a depressive action on the anterior lobe of the pituitary and produces atrophy of the thyroid to the same degree as does hypophysectomy; it also enhances growth and gonadotrophic hormone secretion [9, 10, 11].

### **Parathyroids**

Little evidence is available on the relationship of the thyroid to the parathyroids. The blood calcium level is increased both in hyperthyroidism and in hyperparathyroidism. Thyroid administration or thyrotoxicosis may raise the blood calcium level in hypoparathyroidism.

In relation to the sympathetic nervous system the action of thyroid hormone is stimulant, whereas excess of parathyroid hormone is depressive (see Chapter IV).

### **Adrenals**

Thyroid hormone is one of the most active stimulants of the adrenal cortex [12]. Enlargement of the cortex follows administration of thyroid, whereas thyroidectomy produces atrophy of the cortex, and even inhibits the response of the cortex to injection of pituitary extracts. For example, in myxoedema the excretion of 17-ketosteroids is low.

Thyroid hormone increases the need for cortical hormone; and prolonged stimulation by the thyroid, after having produced hyperplasia of the adrenal cortex, causes exhaustion of the cortex [13]. In hyperthyroidism or Graves's disease the adrenals are smaller and lighter due to the atrophy and lipoid depletion of the cortex [14, 15, 16], whereas the medulla appears to be normal [17]. Graves's disease is accompanied by enlargement of the thymus and hyperplasia of the lymphatic tissue such as is consistently found in adrenal cortical hypoplasia [18, 19, 20]. Selye [51] suggests that some of the adrenal changes in thyrotoxicosis are probably manifestations of a chronic alarm reaction.

The hormone of the adrenal cortex tends to antagonize the effects of the thyroid. In the experimental animal adrenalectomy causes a decrease in the basal metabolism [21, 22]. On the other hand, states of



hyperthyroidism or Graves's disease are favourably influenced by administration of the cortical hormone [23, 24, 25, 26, 27]. According to Goldzieher [28] cortical therapy—with, of course, simultaneous administration of iodine—is a most valuable aid in the medical treatment of hyperthyroidism.

The common coincidence of hyperthyroidism and Addison's disease demonstrates the thyroid-adrenal relationship [29]. In these cases hyperthyroidism may have produced Addison's disease by prolonged stimulation of the adrenal cortex and its subsequent failure. On the other hand, patients suffering from Addison's disease may suddenly develop symptoms of hyperthyroidism due to the thyroid being relieved from the damping effect of the adrenal cortex [30] (see Chapter V).

### **Adrenal Medulla**

The relationship between the thyroid and the adrenal medulla is one of synergism. The thyroidectomized animal does not respond adequately to adrenalin; on the other hand, administration of thyroid hormone increases the pressor and glycaemic response to adrenalin. Both hormones liberate potassium from the tissues.

### **Pancreas**

Thyroid hormone and insulin are mutually antagonistic. Sugar tolerance is often low in Graves's disease and high in myxoedema. Diabetes mellitus is sometimes relieved by thyroidectomy and aggravated by thyroid feeding or spontaneous hyperthyroidism, the excessive thyroid hormone depleting the liver and muscle glycogen.

### **Sex Glands**

Transitory thyroid enlargement is observed at puberty, and during menstruation, pregnancy and lactation.

Arvey and Meyer [5] noted that the B.M.R. rose before menstruation and dropped to its lowest level at the time of bleeding. Interesting observations have been made by Hitchcock and Wardwell [31] studying a group of normal women in whom the basal metabolic rate was determined frequently throughout the menstrual cycle. The metabolic rate was lower during menstruation and midway between periods, but higher pre-menstrually.

Normal pregnancy is accompanied by thyroid enlargement and the basal metabolic rate rises by 20 per cent or 30 per cent during the last few months of pregnancy [32]. After parturition the thyroid undergoes involution and the metabolic rate returns to normal.

The increased thyroid function during pregnancy produces a certain degree of thyroid tolerance. The metamorphosis of tadpoles (*Rana temporaria*) induced by thyroxin can be inhibited by adding blood from a pregnant woman, but it proceeds normally in controls or which blood of a non-pregnant woman is used [33].

Hypothyroidism may delay maturity and retard genital development. In myxoedema libido is decreased; gonadal function is present but low. Subfertility and habitual abortions are frequently associated with either hypo- or hyperthyroidism. Functional uterine bleeding may be accompanied by hypothyroidism.

Administration of thyroid in small doses has produced beneficial results in cases of irregular, excessive or prolonged bleeding associated with a low basal metabolic rate. It is suggested that this effect is due to an increase in the gonadotrophic hormone content of the anterior lobe of the pituitary gland produced by thyroid feeding [35, 36]. Vinchster [37] observed a 50–60 per cent increase in the rate of egg production in hens after the administration of small doses of thyroid. High doses exert a depressive effect on gonadal functions. Thyrotoxicosis is frequently associated with irregular, scanty or completely absent uterine bleeding. Uotila [38] found that in the rat thyroxin produced atrophy of the seminal vesicles.

It is interesting to note that the lower values in the basal metabolic rate observed during the menstrual cycle [31] coincide with the peaks of oestrogen excretion. This suggests that in the normal individual thyroid activity is somewhat decreased when blood and urine oestrogens are high and vice versa. Clinically, oestrogens have been shown to depress thyroid function and lower the basal metabolic rate [39, 40, 41]. The effect of oestrogen on the gonadal system depends on the state of thyroid function. An excess of thyroid increases the threshold of response to oestrogen. More oestrogen is required to induce estrus in the thyroid-fed than in the untreated castrate. Likewise, the threshold of response to gonadotrophic and androgenic hormones is increased in animals with hyperthyroidism [42] (see Chapter VII).

The androgens exert a stimulating effect on thyroid function. Administration of testosterone propionate 25–50 mg. parenterally, three times a week, or of methyl testosterone 30–90 mg. orally every day increases basal metabolism by as much as 30–60 per cent [43].

### Thymus

Patients suffering from hyperthyroidism have an enlarged thymus, whereas thyroidectomy hastens involution of the thymus [44].

Speydel [45] found enlargement of the thymus in tadpoles after thyroid feeding (see Chapter X).

## RELATION OF THE THYROID GLAND TO VITAMINS

### Vitamin A

Clinical observations on the use of thyroid hormone and vitamin A have been most conflicting. According to some workers patients suffering from thyrotoxicosis have been benefited by cod-liver oil, but this result may have been due to the iodine content of the oil.

Support for the view that thyroid hormone stimulates the conversion of carotene to vitamin A is found in the occurrence of night blindness in hypothyroidism, and of a low level of vitamin A in the blood of cretins [47, 48]. In the livers of patients dying from thyrotoxicosis, Moore [50] found larger stores of vitamin A than were present in other human livers, indicating that destruction of vitamin A in the body is not increased by thyroxin.

On the basis of our present knowledge it can thus be concluded:

1. The thyroid stimulates the conversion of carotene to vitamin A but does not increase the bodily requirements of the latter.
2. Vitamin A decreases the effect of thyroxin as a stimulant of metabolism [46] (see Chapter XIV).

### Vitamin B<sub>1</sub> (aneurin, thiamin)

There are striking clinical resemblances between thyrotoxicosis and vitamin B<sub>1</sub> deficiency. Anorexia, diarrhoea, constipation, hypochlorhydria and achlorhydria, tachycardia, enlargement of the heart, dyspnoea, palpitation, oedema, fatigue, muscular pains, lowered muscular strength, neurasthenia, neuritis and disturbances in carbohydrate metabolism are common in both conditions. Means and others [58] have reported improvement of these symptoms in hyperthyroidism by the administration of vitamin B<sub>1</sub>. Williams and co-workers [59] regard the administration of vitamin B<sub>1</sub> as a valuable adjunct in the treatment of thyrotoxicosis. According to these authors the vitamin B<sub>1</sub> deficiency in thyrotoxicosis is due to waste of this substance in the stools, urine and sweat, as well as to excessive combustion of food. It is possible that thyrotoxic patients cannot store as much vitamin B<sub>1</sub> as normal subjects owing to the frequently co-existing hepatic and muscular diseases. One of the authors has routinely prescribed brewers' yeast and vitamin B<sub>1</sub> for all thyrotoxic



patients during the last 4 years. Patients have shown both subjective and objective improvement. Ten  $\mu\text{g.}$  vitamin B<sub>1</sub> are said to detoxicate 50  $\mu\text{g.}$  thyroxin [52].

An accepted clinical sign of vitamin B<sub>1</sub> deficiency is the poor utilization of pyruvic acid in this condition. Williams and co-workers [57] examined a group of 40 thyrotoxic patients and found that nearly 1 of them exhibited high pyruvic acid blood levels (see Chapter XIV).

### **Vitamin C (Ascorbic Acid)**

An interrelation between vitamin C and the thyroid is probable though not definitely proved. In hyperthyroid animals and patients with Graves's disease, the raised blood iodine level is said to be lowered by the administration of ascorbic acid. Presumably the ascorbic acid does not influence the thyroid histologically, but inhibits the thyrotrophic action of the anterior pituitary hormone. Spence and Scowen [54], however, maintain that ascorbic acid does not prevent the production of the goitre caused by feeding rabbits with cabbage. Marine [53], on the other hand, observed that vitamin C prevents thyroid hypertrophy in guinea-pigs injected with the thyrotrophic factor of the anterior pituitary gland. Hayl [55] found histological changes in the thyroid produced by large doses of vitamin C similar to those produced by thyroxin.

### **Vitamin D (Calciferol)**

Vitamin D in doses on the threshold of toxicity raises the B.M.R. by stimulating the thyroid, presumably through the thyrotrophic mechanism of the pituitary.

### **STANDARDIZATION**

The standardization of the thyroid hormone is very unsatisfactory. Since thyroid activity is proportional to its iodine content, the U.K. Pharmacopoeia has adopted as a measure of activity the iodine content of the thyroid substance.

### **Desiccated Thyroid B.P.**

The adopted standard is 0.09–0.11 per cent of iodine.

The U.S.P. product contains 0.17–0.23 per cent of iodine.

### **Thyroxinsodium B.P.**

This contains 61–5 per cent of iodine.

One mg. is equivalent in activity to 0.2 gram of the dried gland and causes an average increase of 2.8 per cent in the B.M.R.

INDOLINGA

### Thyroxin U.S.A.

This contains not less than 64 per cent of iodine.

#### PREPARATIONS FOR CLINICAL USE

Desiccated thyroid is available in tablets of  $\frac{1}{10}$ ,  $\frac{1}{4}$ ,  $\frac{1}{2}$ , 1, 2, 3 and 5 grains.

Thyroxin is available in tablets, in ampoules of 1 and 10 mg. of sterile crystals for intravenous injections, and in solution of 2 mg. per c.c.

#### CLINICAL APPLICATIONS, AND APPROXIMATE DOSAGES

The thyroid hormone has a cumulative effect, the maximum of which for a single dose is reached within about 10 days. Daily administration of the thyroid hormone, therefore, produces its maximum effect within about 20 days, and doses should, for this reason, not be increased in periods of less than 3 weeks.

Beneficial results of thyroid therapy have been claimed for the following conditions: cretinism, myxoedema, hypothyroidism, thyroid dwarfism, obesity, hypogonadism, amenorrhoea, oligomenorrhoea, hypomenorrhoea, functional uterine bleeding, sterility, threatened and habitual abortion, deficient lactation, digestive disturbances, alopecia, trophic disturbances of the nails and teeth, mental torpor, cold extremities, Thompson's disease, Menière's Syndrome, myasthenia gravis, periodic paralysis, furunculosis.

### Obesity

Contrary to the very general belief obesity is not a common symptom of hypothyroidism. In fact, hypothyroidism may exist without producing obesity, and only a small proportion of cases of obesity are associated with hypothyroidism. There has been much controversy on whether desiccated thyroid therapy should be applied in all cases of obesity or only in those in which the aetiological factor has been recognized as hypothyroidism. A number of authors report successful results with thyroid treatment in cases of obesity [61, 62, 63].

Kalb [64] reported that desiccated thyroid alone or in combination with amphetamine sulphate failed to increase the rate of weight loss over that resulting from sub-maintenance diet alone. Whenever it is decided to utilize the calorogenic property of desiccated thyroid, care should be taken to avoid the disturbing effects of increased cardiac activity. One to two grains daily may be given as long as weight loss does not exceed 2 pounds a week. Loss of weight at this rate, however, is usually achieved, without thyroid medication, on a low carbo-

hydrate salt-free diet with reduced intake of fluids alone. A greater rate of weight-loss should not be encouraged.

### **Cretinism**

Timwich and others [65] studied the brain metabolism, before and after administration of desiccated thyroid, in 11 cretins whose ages ranged from 9 to 31. In 8 cretins the average acceleration of cerebral blood-flow was 57 per cent; an average increase of 32 per cent in cerebral metabolism was thus revealed as the result of the administration of thyroid. The most conspicuous result of the treatment was an acceleration of psychological activity.

### **Amenorrhoea, Oligomenorrhoea and Hypomenorrhoea**

Thyroid hormone is effective when the conditions are functional. In such cases they may be associated with obesity and decreased B.M.R.

Desiccated Thyroid:  $\frac{1}{2}$ –1 grain 3 times daily.

Dodds and others [66, 67, 68].

### **Functional Uterine Bleeding**

The treatment is particularly effective when the condition is associated with obesity and low B.M.R.

Desiccated Thyroid:  $\frac{1}{2}$ –1 grain 3 times daily.

### **Sterility in the Female,**

Desiccated Thyroid:  $\frac{1}{2}$ –1 grain 3 times daily.

Winkelstein [69].

Litzenberg recorded good results in 30 per cent of cases when the sterility was associated with a low basal metabolic rate.

### **Threatened Habitual Abortion**

Again, the treatment is most effective when the condition is associated with obesity and hypothyroidism.

Desiccated Thyroid:  $\frac{1}{2}$  grain 3 times daily.

King and others [70, 30].

### **Periodic Paralysis**

Desiccated Thyroid:  $\frac{1}{2}$ –1 grain 3 times daily.

This dosage prevented attacks in 7 patients for the duration of treatment, i.e. one and a half years (Wolf, [34]).

### **Furunculosis**

Desiccated Thyroid: 1 grain daily.

Sixteen patients suffering from furunculosis showed a B.M.R.



below normal. In all cases there was an improvement under thyroid therapy (Barnes [49]).

### BIBLIOGRAPHY

1. VOITKEVITCH, A. A. *Bull. Exper. Biol. & Med. U.S.S.R.*, 7-9, 1943.
2. DEMPSEY, E. C., and ASTWOOD, A. B. *Endocrinol.*, 32, 509, 1943.
3. GOLDZIEHER, M. A., and GORDON, M. B. *Endocrinol.*, 17, 5, 1933.
4. WERNER, A. A. "Endocrinology", H. Kimpton, London, 1942.
5. VON ARVAY, A., and MEYER, H. *Zentralbl. f. Gynäk.*, 56, 194, 1932.
6. BEAUMONT, G. E., and ROBERTSON, J. D. *Brit. Med. J.*, 4315, 356, 1943;  
*Brit. Med. J.*, 4322, 578, 1943.
7. MACKAY, E. M., and SHERRILL, J. W. *J. Clin. Endocrinol.*, 3, 462, 1943.
8. ROGOWITSCH, N. *Beitr. Z. Path. Anat. U.Z. Allg.*, 4, 452, 1888.
9. KOSCHINSKY, G. *Arch. F. Exper. Path. U. Pharmakol.*, 170, 510, 1933.
10. MARINE, D. *J.A.M.A.*, 104, 2250, 1935.
11. UOTILA, U. U. *Endocrinol.*, 26, 129, 1940.
12. HOSKINS, R. G. *J.A.M.A.*, 55, 1725, 1910; *Arch. Int. Med.*, 17, 584,  
1916.
13. ZWEMER, R. L. *Am. J. Physiol.*, 79, 658, 1927.
14. LANDAU, M. "Die Nebennierenrinde", Jena, 1915.
15. WEGELIN, C. "Henke-Lubarsch, Hdbuch. d. Path. Anat.", VIII,  
Springer, 1926.
16. RAUTMANN, H. *Mitt. Grenzgeb. Chir. U. Med.*, 28, 489, 1915.
17. GOLDZIEHER, M. A. "The Adrenals in Health and Disease", p. 642,  
F. A. Davis Co., Philadelphia, 1944.
18. MARINE, D. *J. Am. Med. Sc.*, 180, 767, 1930.
19. MATTI, H. *Deutsch. Zeitschr. f. Chir.*, 10, 1893.
20. CAPELLE, W. *Beitr. Kl. Chir.*, 59, 353, 1908.
21. MARINE, D., and BAUMANN, E. J. *Am. J. Physiol.*, 57, 135, 1921; 59,  
353, 1922.
22. SCOTT, W. J. M. *J. Exper. Med.*, 36, 199, 1922.
23. MARINE, D. *J. Am. Med. Sc.*, 180, 767, 1930.
24. BRAM, I. "Exophthalmic Goiter", Mosby, St. Louis, 1936.
25. RICHARDSON, J. A. *Acta Med. Scand.*, 98, 581, 1939.
26. OEHME, C. *Kl. Wschr.*, 15, 512, 1935.
27. THADDEA, S. "Die Nebennierenrinde", Leipzig, Thieme, 1936.
28. GOLDZIEHER, M. A. "Adrenal Glands in Health and Disease", p. 644,  
F. A. Davis Co., Philadelphia, 1944.
29. *Ibid.*, p. 645.
30. BOLS, J. K. *South. Med. J.*, 30, 637, 1937.
31. HITCHCOCK, F. A., and WARDWELL, F. A. *J. Nutr.*, 2, 203, 1929.
32. SANDIFORD, I., and WHEELER, T. *J. Biol. Chem.*, 62, 329, 1924.
33. SAX, M. G. *J. of Physiol. of the U.S.S.R.*, 1, 144, 1940.
34. WOLF, A. *N.Y. State J. of Med.*, 43, 1951, 1943.

35. EVANS, H. M., and SIMPSON, M. E. *Anat. Rec.*, **45**, 215, 1930.
36. VAN HORN, W. M. *Endocrinol.*, **17**, 152, 1933.
37. WINCHESTER, C. F. *Endocrinol.*, **24**, 697, 1939.
38. UOTILA, U. U. *Endocrinol.*, **26**, 129, 1940.
39. GESSLER, C. *Arch. Internat. de Pharmacodyn et de Therap.*, **54**, 263, 1936.
40. SHERWOOD, T. C., SAVAGE, M., and HALL, J. F. *Am. J. Physiol.*, **105**, 241, 1933.
41. FARBMAN, A. A. *J. Clin. Endocrinol.*, **4**, 17, 1944.
42. SMELZER, G. K. *Anat. Rec.*, **73**, 273, 1939.
43. THOMPSON, W. O. *J.A.M.A.*, **125**, 15, 1941.
44. CHIODI, H. *Rev. Soc. Argent. de Biol.*, **14**, 322, 1938.
45. SPEYDEL, C. C. *Am. J. Anat.*, **37**, 141, 1926.
46. BICKNELL and PRESCOTT. "The Vitamins in Medicine", p. 37, Heine-mann, London, 1942.
47. WOHL, M. G., and FELDMANN, J. B. *Endocrinol.*, **24**, 389, 1939.
48. WENDT, H. *Munchen. Med. Wchnschr.*, **82**, 1679, 1935.
49. BARNES, B. *J. Clin. Endocrinol.*, **3**, 243, 1943.
50. MOORE, T. *Biochem. J.*, **31**, 155, 1937.
51. SELYE, H. *J. Clin. Endocrinol.*, **6**, 117, 1946.
52. SURE, B., and BUCHANAN, K. S. *J. Nutr.*, **13**, 513, 1937.
53. MARINE, D., BAUMANN, E. J., and ROSEN, S. H. *Proc. Soc. Exper. Biol. & Med.*, **31**, 870, 1934.
54. SPENCE, A. W., and SCOWEN, E. F. *Biochem. J.*, **29**, 562, 1935.
55. HEYL, J. G. *Acta brev. Neerland*, **4**, 12, 1934.
56. BEHRMAN, S. *Proc. Roy. Soc. Med.*, **38**, 665, 1945.
57. WILLIAMS, R. K., and KENDALL, E. C. (Mayo Clinic). *Arch. Int. Med.*, **72**, 185, 1943.
58. MEANS, J. H., HERTZ, S., and LERMAN, J. *Am. Int. Med.*, **11**, 429, 1937.
59. WILLIAMS, R. H., EGANA, ENRIQUES, ROBINSON, P., ASPEN, S. P., and DUTOIT, CHAS. (Boston). *Arch. Int. Med.*, **92**, 353, 1943.
60. BRUNN, E. *Acta Med. Scandinav.*, **121**, 13, 1945.
61. LYON, D. M., and DUNLOP, D. *Quart. J. Med.*, **1**, 331, 1932.
62. BAYER, L. M., and GRAY, H. *Am. J.M. Sc.*, **189**, 86, 1935.
63. HANDELSMAN, M. B., and GORDON, N. R. *J. Clin. Endocrinol.*, **1**, 612, 1941.
64. KALB, S. W. *J. Med. Soc. New Jersey*, **39**, 47, 1942.
65. HIMWICH, H. E., DARBY, C., FAZEKAS, J. F., HERRLICH, H. C. *Am. J. Psychiat.*, **98**, 489, 1942.
66. DODDS, E. C., and ROBERTSON, J. D. *J. Obst. & Gynec. Brit. Emp.*, **46**, 213, 1939.
67. FLUHMAN, C. E., and MURPHY. *Am. J. Obst. & Gynec.*, **42**, 656, 1941.
68. COLLINS, R. M. *Iowa State Med. J.*, **31**, 576, 1941.
69. WINKELSTEIN, P. R. *Am. J. Obst. & Gynec.*, **14**, 1, 1940.
70. KING, E. L., and HERRING, J. S. *J.A.M.A.*, **113**, 1300, 1939.

## PARATHYROID HORMONE

THE parathyroid glands in the human subject are usually found on the dorsal surface of the thyroid gland. They are generally four in number, yellowish-red to brown-red in colour, and vary in size from 5 mm. to 15 mm. long and 2 mm. to 3 mm. broad and thick. They are very vascular and are each supplied by a special arteriole from the thyroid artery. The parathyroid glands are innervated by the laryngeal nerve. In transplantation experiments, however, it has been found that the gland can function without any nerve connections.

## PHYSIOLOGY

The parathyroid glands play an important part in calcium and phosphorus metabolism. About 99 per cent of calcium is deposited in the form of a calcium phosphate-calcium carbonate compound in the bones and teeth. An additional small amount is carried by the body fluids. The normal value for serum calcium is 10.5 mg. per 100 c.c., with  $\pm 1$  as the range of normal variation. However, cases of proved hyperparathyroidism with normal serum calcium levels are reported [1]. A continuous deposition and absorption of calcium takes place in the bones; but according to Albright [2] no such metaplasia of tissue occurs in the teeth, and dental decay during pregnancy is not due to mobilization of calcium from the tooth to the blood-stream. Spira [3] observed mottled teeth in the rat after parathyroidectomy.

Phosphorus is present in the bones and teeth in large amounts, the phosphorus-calcium ratio being 1:2. The normal serum phosphorus level for adults is 3.5 mg., with  $\pm 0.5$  mg. as the range of normal variation.

Prolonged administration of parathyroid hormone induces hypercalcaemia, hypophosphataemia and increased excretion of calcium and phosphorus. If parathyroid hormone administration is discontinued, there follows an immediate decrease in the phosphorus excretion in the urine and an increase in the serum phosphorus level with a simultaneous decrease in the serum calcium level followed by diminished excretion of calcium in the urine.

Neufeld and Collip [4] suggest that the primary action of the parathyroid hormone is not directly on the bone but on the excretion of phosphate by the kidneys. Depression of the value for serum phosphorus measured as inorganic phosphate is the rule in cases without gross impairment of renal function. The serum alkaline phosphate level is elevated in proportion to the degree of involvement of bone.



The bone changes in hyperparathyroidism appear to be an index more of the duration of the disease than of its severity [1]. It has been observed that the severity of tetanic manifestations is not directly proportional to the blood-calcium level; and this has led to the suggestion that both the lowered serum calcium level and tetany are caused by the retention of inorganic phosphorus [5].

#### HYPERPARATHYROIDISM (VON RECKLINGHAUSEN'S DISEASE)

Increased parathyroid activity may occur as primary hyperfunction or secondarily to a negative calcium balance.

#### **Primary Hyperparathyroidism**

This is due to single or multiple adenomas of the glands, or to hypertrophy of all the parathyroid tissue. The serum-calcium level is characteristically high, up to 18 mg., in severe conditions, and the serum-phosphorus level is invariably low, 3.1 mg. or lower, unless renal damage is present [6]. The excretion of both calcium and phosphorus in the urine is increased. In the presence of increased bone metaplasia, the serum-phosphatase level is increased, reaching 20 to 30 Bodansky Units in severe forms.

The mechanism of parathyroid action is essentially a lowering of the renal threshold for phosphates. The increased urinary phosphate excretion causes a fall in serum phosphate. According to Albright and others [2, 7], the serum, being less saturated with calcium phosphate, develops an increased requirement for this compound. As a result of this, bone mineral is mobilized, unless the patient ingests as much calcium and phosphorus as is lost in the urine. Calcium is less rapidly excreted in the urine than phosphate and thus produces a higher blood-calcium level, with a tendency to deposit calcium in the soft tissues.

Another school of thought [8] contends that liberation of calcium from the body stores is attributable to the direct action of the parathyroid hormone on the bones. Recent investigations [9], however, showed no direct action of the parathyroid hormone on the bone when femur fragments of a 9-16 days' old chick embryo were explanted into plasma to which the parathyroid hormone was added directly, or which was saturated with parathormone by injecting a cock with the hormone for 10 days before obtaining the plasma. Plasma prepared by the injection of a single large dose of parathyroid hormone into the cock induced definite signs of bone absorption with

the formation of osteoclasts in femur fragments of the chick embryo. Plasma activity ceased one day later.

Hyperparathyroidism usually produces definite changes in the bones, with generalized osteitis fibrosa cystica in the later stages. There is a diminution in the amount of bone tissue due to increased bone absorption, and the number of osteoclasts is increased. Since no fundamental disorder of bone repair processes exists, the number of osteoblasts is also increased. This marked augmentation in the metaplasia of bone results in increased serum phosphatase, which in the absence of hepatic disease is an index of osteoblastic activity [6]. The bone matrix which is laid down by the osteoblasts is calcified and the stroma of the bone marrow is also increased, causing fibrosis. The resulting hypercalcaemia leads to decreased muscular excitability and inhibition of gastric secretion. Increased excretion of calcium and phosphate in the urine predisposes to the formation of calcium phosphate or calcium oxalate urinary calculi [10]. Intensive calcification of the renal interstitial tissues may occur with complete blockage of Bowman's capsules and uriniferous tubules with calcium salts. Acute renal impairment is characterized by a failure to excrete phosphates, which results in a high serum-phosphorus level. The negative calcium balance thus produced is compensated by a further decalcification of bones, causing a high serum-calcium level. At this stage, a precipitation of calcium phosphates into the tissues may occur and result in chemical death. It is suggested that this condition, described as "parathyroid poisoning" [6], may be prevented by keeping patients with severe hyperparathyroidism on a low calcium diet until there is no danger of parathyroid poisoning.

The incidence of the major initial symptoms of hyperparathyroidism is set out in the following figures [11]:

Pain in back or extremities . . . . .	72 per cent
Muscular weakness . . . . .	22 per cent
Pathological fractures . . . . .	28 per cent
Bone swellings . . . . .	26 per cent
Gross deformities . . . . .	19 per cent
Disturbances of gait . . . . .	24 per cent
Renal symptoms, such as polyuria, polydipsia and colic . . . . .	10 per cent
Various gastro-intestinal symptoms . . . . .	2-8 per cent
Renal lithiasis [1] . . . . .	60 per cent

### Secondary Hyperparathyroidism

This may be due to vitamin D deficiency (rickets and osteomalacia) or to renal impairment.

Vitamin D deficiency causes failure of calcium absorption from the gastro-intestinal tract. This results in a low serum-calcium level compensated for by increased parathyroid function, which in turn restores the normal calcium level and lowers the serum-phosphorus level by increasing the urinary excretion of phosphate. The result is the usual finding of a normal calcium and low serum-phosphorus level [12].

Renal insufficiency associated with retention of serum phosphorus produces a negative calcium balance, which in turn induces increased parathyroid function producing decalcification of bones and a high serum-calcium level.

#### HYPOPARATHYROIDISM

Hypoparathyroidism is associated with a low calcium and high phosphorus level in the serum. Hypocalcaemia causes increased neuromuscular excitability, and in its more severe form the symptom-complex known as tetany. The density of the bones is increased and calcification of brain tissue is a not infrequent finding [13].

In addition to perivascular cerebral calcification, hypoparathyroidism may be manifested in lenticular cataract, dryness and brittleness of the hair and nails, dryness of the skin, muscular weakness, constipation, insomnia, psychoasthenia and general slowing down of the mental processes. Chronic latent hypoparathyroidism may produce a variety of vague misleading symptoms. Numbness and tingling, a feeling of stiffness, and a slight twitching of the muscles of the face or extremities may be important early signs.

#### THE PARATHYROID IN RELATION TO OTHER GLANDS

(see Fig. 1)

##### **Anterior Pituitary Gland**

It has been suggested that the anterior pituitary gland stimulates parathyroid function. In hypopituitarism, however, there is no clinical evidence of hypoparathyroidism [2].

##### **Thyroid Gland**

Removal of either the thyroid or parathyroid gland is followed by hypertrophy of the other. Thyroxin raises the serum-calcium level in hypoparathyroidism. Graves's disease, however, may be accompanied by either tetany or hyperparathyroidism, and parathyroid tetany may be associated with myxoedema or hyperthyroidism (see Chapter III).



### **Adrenal Medulla**

In parathyroid insufficiency, owing to a decreased blood-calcium level, the response to adrenalin is diminished. Tetanic convulsions, however, increase the discharge of adrenalin. The injection of parathormone, although increasing the blood calcium, does not substantially alter the response to adrenalin [14]. According to Zondek [15], increased parathyroid activity sometimes inhibits the function of the adrenal medulla.

### **Adrenal Cortex**

The relation of the parathyroid to the adrenal cortex is apparently one of antagonism. Both hyperparathyroidism and acute adrenal insufficiency are characterized by a loss of sodium chloride and dehydration followed by oliguria and azotemia (see Chapter V).

### **Pancreas**

The parathyroid hormone appears to stimulate insulin secretion [15].

### **Oestrogens**

M. and R. Silberberg [16] demonstrated that prolonged administration of oestrogens produces changes similar to those of osteitis fibrosa. The increase of blood calcium and the increased bone metaplasia after oestrogen injections suggests that the oestrogen acts through the parathyroid gland [17].

### **Gonadal System**

Latent tetany is often activated during menstruation, pregnancy, the puerperium and lactation. Hyperparathyroidism may cause irregular menstruation or amenorrhoea; on the other hand, the administration of parathyroid extract has given beneficial results in excessive uterine bleeding [18].

In the male, hyperparathyroidism is frequently accompanied by loss of libido and potency.

### **THE PARATHYROID AND VITAMIN D**

Both vitamin D and the parathyroid hormone regulate calcium and phosphorus metabolism. Each exerts its action in its own way, and the effects produced depend on the quantitative relationship of the two substances. Clinically, the mode of action changes according to the dosages applied. There appears to be no direct relationship, for the vitamin exerts its effects even in parathyroidectomized animals.

The action of vitamin D in physiological doses consists in mobilizing phosphorus from the tissues and aiding its combination with calcium by converting organic phosphorus into an inorganic form [19]. Its effect on calcium is to increase its absorption from the gastro-intestinal tract. Parathyroid hormone lowers the level of inorganic phosphorus by diuresis and tends to cause hypercalcaemia by the mobilization of calcium salts from the bones.

Excessive amounts of vitamin D lead, in addition to the effects of small doses, to increased excretion of phosphate by the kidneys [20]. The lowered serum-phosphate level brings about the mobilization of phosphorus and calcium from the bones, with dissolution of the trabeculae but without the replacement of these by fibrous tissue. Calcification of the soft tissues (kidneys, lungs, thyroid) occurs [6]. Excessive doses of parathyroid hormone act on the bone by decalcification and replacement with fibrous tissue and giant cells. Clinically the difference in action is important, since rickets is cured by small (anti-rachitic) doses of vitamin D, but made worse by large (calcaemic) doses and by parathormone. In hypoparathyroidism anti-rachitic doses of vitamin D are ineffective, and large amounts have to be employed. The initial beneficial effect of parathormone wears off, but the effect of vitamin D does not. Milk, though rich in calcium, is contraindicated in the treatment of hypoparathyroidism because it is also rich in phosphorus (see Chapter XIV).

## STANDARDIZATION

### Parathyroid Hormone

The parathyroid hormone was formerly standardized in terms of Collip Units, defined as one-hundredth of the amount of hormone required to cause an average increase of 5 mg. of calcium per 100 c.c. of blood serum within 15 hours in dogs weighing 20 kg. The recently adopted International Unit represents one-fifth of the Collip Unit. The method of assay has not proved altogether satisfactory since dogs differ in their response and even the same dog responds differently at different times.

### Vitamin D

Vitamin D is standardized in terms of its anti-rachitic potency, which is determined by the degree of decalcification prevented in rats fed on a rachitogenic diet, or by the calcification produced with vitamin D in rachitic rats. Calcification is measured by the deposition of bone,

or by the weight of the femoral ashes, or radiographically. The standard vitamin-D preparation is used in every assay as a control.

The International Unit of vitamin D is the amount of vitamin D equal in anti-rachitic potency to 1 mg. of the international standard solution. This International Unit is the same as the United States Unit.

1 I.U. of vitamin D is equivalent to 0.025 micrograms of crystalline vitamin D<sub>2</sub> (Calciferol).

1 mg. of Calciferol is equivalent to 40,000 I.U. of vitamin D.

1 c.c. of Dihydrotachysterol is equivalent to 10 mg. of Calciferol, or 400,000 I.U. of vitamin D.

#### PREPARATIONS FOR CLINICAL USE

##### **Parathyroid Hormone**

The parathyroid hormone, although very active in raising the blood-calcium level, has not found wide application in the treatment of parathyroid insufficiency. There is a long latent period before the hormone raises the serum calcium, and repeated injections cause some degree of immunity; 1 c.c. of the hormone contains 100 I.U., or 20 dog units.

About 100 I.U. of the hormone will raise the blood-serum calcium in tetany from 5 to 8 mg. per 100 c.c.; and 100–300 I.U. will raise it to normal levels for the period of 8–18 hours following the injection.

##### **Vitamin D**

This is a general term, used for the naturally occurring vitamin D and the substances obtained by irradiating ergosterol.

##### **Vitamin D<sub>1</sub>**

This name is no longer used, since it was given to a substance which was later found to be a mixture of calciferol and lumisterol (intermediate irradiation product).

##### **Vitamin D<sub>2</sub> or Calciferol**

This does not occur naturally but is manufactured by irradiation of ergosterol (ergosterol—lumisterol—tachysterol—calciferol) and is available in pure crystalline form.

1 mg. of Calciferol is equivalent to 40,000 I.U. of vitamin D or to 0.1 c.c. of Dihydrotachysterol.



*Range of Dosage*

Anti-rachitic dose: About 1,200 I.U. of vitamin D daily.

Calcaemic dose: 60,000–200,000 I.U. of vitamin D daily.

**Vitamin D<sub>3</sub>**

This is the form of naturally-occurring vitamin D which preponderates in fish-liver oils. It is formed by activation of the animal sterol, 7-dehydrocholesterol.

**Viosterol**

Viosterol is the non-proprietary name adopted by the Council on Pharmacy and Chemistry of the American Medical Association for all acceptable preparations of irradiated ergosterol [21]. It consists of about 50 per cent of calciferol, the rest being mainly lumisterol and tachysterol. It is standardized in terms of vitamin D activity, so that the content of lumisterol and tachysterol, which have little or no anti-rachitic effect, are not taken into account.

**Dihydrotachysterol (A.T.10)**

This is prepared from tachysterol, one of the products of irradiation of ergosterol. Dihydrotachysterol is available in the form of a 0.5 per cent solution in oil. It is effective by mouth but not subcutaneously or intramuscularly. The effect of a single dose appears after 48 hours, and a maximum blood-calcium level is obtained about 10 days after starting its administration [22]. The substance does not cause immunity. Its action is said more closely to resemble that of the parathyroid hormone than of pure vitamin D. It increases phosphate excretion and, in addition, is believed slightly to increase the retention and absorption of calcium. Clinically, however, calciferol, administered in the equivalent dosage, has been shown to exert the same effect.

1 c.c. of Dihydrotachysterol is equivalent to 400,000 I.U. of vitamin D

to 10 mg. of Calciferol.

*Range of Dosage*

Initial dose: 1–4 c.c. daily until calcium appears in the urine, and thereafter

Maintenance dose: 0.3–1.0 c.c. daily.

Dihydrotachysterol has, like the parathyroid hormone, a cumulative effect, and serum calcium analyses should be made every two or

three weeks. For this a urine test with Sulkowitch's Reagent is carried out. This consists of:

Oxalic acid . . . . .	2.5 grams
Ammonium oxalate . . . . .	2.5 grams
Glacial acetic acid . . . . .	5.0 c.c.

dissolved in water and made up to a volume of 150 c.c. Five c.c. of this reagent mixed with an equal volume of urine produces the immediate precipitation of any calcium present. The urine must be acid, or, if not, made acid before the test. If the precipitate does not form, it indicates that the blood-calcium level is below normal, which is 9.5–11 mg. Formation of a fine white cloud indicates the presence of a moderate amount of calcium in the urine, and probable a normal level in the blood. If the precipitate is heavy, giving a milky appearance, large amounts of calcium are present and there is danger of hypercalcaemia.

The Sulkowitch test provides a rough estimate of the existence or non-existence of excessive urinary excretion of calcium. Considered alone, it is not diagnostic of hyperparathyroidism.

## CLINICAL APPLICATIONS AND APPROXIMATE DOSAGES

### Primary Hyperparathyroidism

No satisfactory medical treatment exists. Fluids should be given in large amounts and milk should be withdrawn from the diet to avoid nephrocalcinosis and parathyroid poisoning. The surgical removal of the over-active glands is indicated. Some beneficial results have been obtained by X-ray therapy [23].

### Secondary Hyperparathyroidism

Rickets:

Vitamin D 700–1,000 I.U. daily [24].

Vitamin D about 1,200 I.U. daily for infants with moderately severe rickets [19].

Vitamin D up to 1,200 I.U. daily can be given as cod-liver oil; higher doses require one of the concentrated fish-liver oils [19]. Higher doses are usually not needed except for infants in whom weakness and collapse of the ribs threatens death from pulmonary infections, in which case vitamin D 50,000 I.U. may be given daily. Repeated blood examinations and X-rays are indicated [19].

Osteomalacia:

Vitamin D 3,000 I.U. and more daily.

Calcium phosphate 30 grains 3 times daily.

Plenty of milk, cheese and fish [19].

### Hypoparathyroidism

Initial dose:

Parathyroid hormone:

100–150 I.U. 2 or 3 times within the first 24 hours.

Calciferol:

1,500,000 I.U. daily [25]—3,000,000 I.U. daily [26] for 2 weeks.

Dihydrotachysterol:

1–4 c.c. daily are commonly given until the serum-calcium level rises to 9–10 mg. per 100 c.c. and the symptoms of tetany subside [27]. Higher initial doses have been given without toxic reactions. The maximal blood-calcium level is attained after about 10 days from the beginning of administration.

Maintenance dose:

Parathyroid hormone:

The dose is decreased after 24 hours as indicated by the condition of the patient.

Calciferol:

1.5–10 mg. (60,000–400,000 I.U.) are given daily as extreme dosage limits.

3–5 mg. (120,000–200,000 I.U.) daily are the more commonly prescribed doses [27].

Dihydrotachysterol:

0.15–1.0 c.c. daily are given as the extreme dosage limits.

0.3–0.5 c.c. daily are the more commonly prescribed doses [27].

During the second half of pregnancy as much as 3 c.c. have been given daily as a maintenance dose [28, 33].

Vitamin D is advocated in a number of other conditions in which raised blood-calcium level is held to be an advantage.

Fractures: Vitamin D 600 I.U. daily [19].

Arthritis: Vitamin D 150,000–200,000 I.U. daily [29].

Psoriasis: Vitamin D 300,000–500,000 I.U. daily [30, 31].



Scleroderma: Vitamin D 200,000–300,000 I.U. daily [31].

Asthma and Hay Fever: Vitamin D 100,000–300,000 I.U. daily [32].

## BIBLIOGRAPHY

1. ALEXANDER, H. B., PEMBERTON, J., KEPLER, E. J., and BRODERS, A. C. *Am. J. Surg.*, **65**, 157, 1944.
2. ALBRIGHT, F. "Glandular Physiology and Therapy", *Am. Med. Assn.*, 1942.
3. SPIRA, L. J. *Hyg.*, **42**, 500, 1942.
4. NEUFELD, A. H., and COLLIP, J. B. *Endocrinol.*, **30**, 135, 1942.
5. GREENWALD, I. *J. Biol. Chem.*, **67**, 1, 1926.
6. COVEY, G. W., and WHITLOCK, H. H. *Ann. Int. Med.*, **25**, 508, 1946.
7. ELLSWORTHY, R. J. *Clin. Invest.*, **11**, 1011, 1932.
8. THOMSON, D. L., and COLLIP, J. B. *Physiol. Rev.*, **12**, 309, 1932.
9. JAFFE, H. L. *Arch. Path.*, **16**, 63, 1933.
10. RUNJANTZEW, A. W., and BEREZKINA, L. F. *Compt. Rend. de l'Acad. des Sci. de l'U.R.S.S.*, No. 6, 1944.
11. ALBRIGHT, F. *New England J. Med.*, **209**, 476, 1933.
12. GUTMAN, SWANSON and PARSONS, quoted by BODANSKY, M., and BODANSKY, O. "Biochemistry of Disease", The Macmillan Co., New York, 1941.
13. ALBRIGHT, F., and SULKOWITCH, H. W. *J. Clin. Invest.*, **17**, 305, 1938.
14. EATON, L. M., and HAINES, S. F. *Proc. Staff Meet. Mayo Clin.*, **14**, 48, 1939.
15. CSEPEAI, K., and FERNABH, J. *Ztschr. Ges. Exp. Med.*, **60**, 619, 1928.
16. ZONDEK, H. "Diseases of the Endocrine Glands", Edward Arnold & Co., London, 1944.
17. SILBERBERG, M. and R. *Arch. Path.*, **28**, 340, 1939; *ibid.*, **31**, 85, 1941.
18. BACH, E. *Klin. Wchnschr.*, **16**, 218, 1937.
19. HARTLEY, E. C. *Am. J. Obst. & Gynec.* **21**, 725 1931; *ibid.*, **27**, 253, 1934.
20. BICKNELL, B., and PRESCOTT, F. "The Vitamins in Medicine", p. 458, W. Heinemann, London, 1942.
21. HARRISON, H. E., and HARRISON, H. C. *J. Clin. Invest.*, **20**, 48, 1941.
22. "New and Non-official Remedies", Chicago Am. Med. Assn., 1940.
23. GROLLMAN, A. "Essentials of Endocrinology", J. B. Lippincott Co., Philadelphia, 1941.
24. MERRITT and CAULK. *Radiology*, **35**, 477, 1940.
25. HELFET. *Brit. J. Surg.*, **27**, 651, 1939.
26. PARK, E. A. *J.A.M.A.*, **115**, 370, 1940.
27. HIMSWORTH, H. P., and MAIZILS, M. *Lancet*, **1**, 453, 1940.
28. STECK, I. E., DEUTSCH, H., REED, C. I., and STRUCK, H. C. *Ann. Int. Med.*, **10**, 951, 1937.

27. MCLEAN, F. C. "Glandular Physiology and Therapy", Am. Med. Assn., Chicago, 1941.
28. CURTIS, J. K. *M. Clin. North America*, **24**, 833, 1940.
29. REED, A. M., STRUCK, H. C., and STECK, I. E. "Vitamin D", Chicago, 1939.
30. BRUNSTING, L. A. *Proc. Staff. Meet., Mayo Clinic*, **13**, 280, 1938.
31. MAYNARD, M. T. R. *Arch. Dermat. Syphil.*, **41**, 842, 1940.
32. RAPPAPORT, B. L., and REED, C. I. *J.A.M.A.*, **101**, 105, 1933.
33. SCHWARTZ, H. A., CURTIS, J. K., and LICHTENSTEIN, J. V. *Am. J. Obst. & Gynec.*, **41**, 697, 1941.

## THE SUPRARENAL GLAND HORMONES

THE adrenals in man are two flat bodies situated above the upper pole of the kidney and attached to it by connective tissue. They are enclosed in a firm fibrous capsule which is covered by ample fatty tissue. Their shape and size are found to vary considerably, particularly in different age groups; and their weight and size are much influenced by sex. The decrease in size which occurs with advancing years is not accompanied by decrease in weight. Each gland usually weighs 6–8 grams.

In children, the adrenal glands are slightly heavier in the female; but during adolescence, up to the age of sexual maturity, the male adrenals are heavier. After sexual maturity a more rapid growth of the glands occurs in the female and they become markedly heavier [1]. After the thirtieth year, the male adrenals are again the heavier and so remain for the rest of life.

The rich blood supply of the adrenals derives from the superior suprarenal, middle suprarenal and inferior suprarenal arteries. Each gland is surrounded by a rich venous plexus which communicates with the renal plexus. Blood passes through the adrenals in an amount equivalent to six times the weight of the glands each minute. A rich lymphatic network is also present. The nervous control of the glands is sympathetic for the medulla, which functions as a modified sympathetic ganglion, and parasympathetic for the cortex. The fact that the parasympathetic nerve fibres have vascular connections only—i.e. none directly with the gland—suggests that any nervous control of adrenal cortical secretion is probably exercised through the blood-vessels. MacFarland [2] recently demonstrated, using transplantations of normal denervated adrenals in 95 mature albino rats, that cortical activity is not under direct neural control, whereas the medulla is innervated by secretory fibres.

## ADRENAL CORTEX

Microscopically, the cortex exhibits three strata: the glomerular (external zone), the fasciculate and the reticulate (adjacent to the medulla). No sharp demarcation between them can be noticed. Bennett [3] distinguishes four zones of the cortex: pre-secretory, secretory, post-secretory and senescent.

The specific cells of the cortex contain typical mitochondria and are characterized by the presence of lipoid granules. In the reticulate



zone, pigment granules are responsible for the brownish-yellow colour. Acute demand causes discharge of material from mature cells, whereas prolonged demand stimulates formation of new cells. Stimulation of the cortex by cold or formaldehyde produces enlargement of the secretory zone with decrease of its lipid content which eventually may become completely exhausted [4]. Adrenal enlargement, associated with depletion of lipid or reversal of lipid pattern, has been found in association with inflammatory disorders, cachexia, pemphigus and protracted emesis [5]. Cortical enlargement, with an increased amount of lipid, has been encountered in cases of hypertension; the change was most striking when hypertension was associated with primary vascular disease [5]. Hypertrophy of the cortex may be due to increased physiological storage of lipoids; it occurs in pregnancy or may be congenital, as in almost all genetically female pseudo-hermaphrodites.

The lipid globules are often surrounded by a ringlike film of ascorbic acid, which seems in some unknown manner to be concerned with the elaboration of the lipid material [6]. The ascorbic acid deposits of the cortex can be demonstrated by reduction of silver nitrate [7]. The substance contained in the lipid droplets has recently been identified as a ketosteroid [8].

*The "X" Zone.*—In the mouse adrenal there is a zone immediately adjacent to the medulla and containing an outer and inner part. The outer part is permanent, whereas the inner (referred to as the "X" zone) is transitory [9]. In the male this "X" zone disappears in about 38 days. In the female it continues to grow until fatty changes set in, leading to disintegration in about 80 days, and is replaced by a connective tissue septum. It has been found that the "X" zone contains ascorbic acid [10].

The cells of the "X" zone are small and more intensely stained than the rest, and its cytoplasm gives a fuchsinophil reaction.

By means of Mason's stain, the post-secretory and senescent zones appear quite broad in the adult male animal, and show intense fuchsinophilia [10]. The same zones are much narrower in the immature male or in the female, and stain a definite green. In fowls, the fuchsinophil reaction of the cytoplasm characteristic of the "X" zone is demonstrable in cells scattered throughout the cortex.

The cortical cells of male foetuses show a fuchsinophil substance from the ninth to the twentieth week, with a maximum intensity between the fifteenth and seventeenth weeks. It is distributed more or less equally throughout the cells in all areas, with no suggestion of definite zoning. In the female, fuchsinophil material is present

from 11 to 15 weeks, with a maximum intensity at 14 weeks [11, 12]. On the assumption that the presence of the fuchsinophil material is related in some way to the presence of androgen, it is suggested that there occurs a short but definite "male phase" in the normal development of the female foetus, representing a short but rapidly terminated period of potential bisexualism [12].

Recent studies of the distribution of ascorbic acid in the cortex have shown that the "X" zone is but a part of the regular cortex and not a separate tissue. This accords with the observation that the cortex has androgenic properties even in species which do not have "X" zones [10]. Broster and others [12] obtained the characteristic fuchsinophil reaction in 34 out of 36 cases of adrenal virilism after adrenalectomy. The authors relate this fuchsinophil material to the masculinizing properties of the adrenal cortex, but they refrain from the definite claim that this substance is actually the male hormone. However, in most cases, the biological test for the presence of androgen showed a definite correlation between the presence of androgen and of fuchsinophil material, though the results did not always agree exactly.

In cases of clinical virilism associated with simple cortical hyperfunction, the fuchsinophil material of the cortical cells appears as a granular deposit in the cytoplasm, chiefly in the zona reticularis. These cells may be regarded as the most differentiated of the cortical cells, both histologically and functionally. The small amount of fuchsinophil material present in the normal adrenal gland is generally localized in these cells of the zona reticularis too.

From the normal adrenal Reichstein [13] isolated a sterol with androgenic properties, which he named andrenosterone and declared to be distinct from the known androgens. Marrian and Butler [14], from the adrenal cortex, isolated pregnanetriol ( $C_{21}H_{36}O_3$ ), a chemical compound allied to the corpus luteum series. From pregnanetriol, they further isolated isoandrosterone, a substance specific for virilism.

#### CORTICAL EXTRACTS

Most of the methods for extracting cortical compounds depend upon repeated distribution between water and benzene which divides the extract into two fractions.

The main constituents of the benzene fraction are:

Compound A (Dihydrocorticosterone);

Compound B (Corticosterone), desoxycorticosterone, progesterone and other related substances.



The main constituents of the aqueous fraction are:

Kendall's Compound E (17-hydroxy-11-dehydrocorticosterone) and Compound F (hydroxycorticosterone).

The potency of the crude extract is considerably greater than that of the sum total of these four constituents.

Reichstein and Euq [15] isolated 11-hydroxyisoandrosterone, which, in the capon's comb, has about one-thirtieth of the activity of androsterone. Pfiffner and North [16] isolated 17-hydroxyprogesterone, which showed androgenic activity in the castrate rat. Progesterone has been isolated by Beall and others [17], and oestrone by Beall [18]. Desoxycorticosterone has about one-tenth the progesterone-like activity of progesterone.

Kendall's Compound E (17-hydroxy-11-dehydrocorticosterone) is a pure crystalline compound with a high glycogenic activity. On the basis of a liver glycogen test it was found that 11-dehydrocorticosterone was one-third as active as 17-hydroxy-11-dehydrocorticosterone [211]. According to the cold-protection test 11-dehydrocorticosterone is one-third, corticosterone one-twelfth, and 11-desoxycorticosterone acetate one-thirteenth as active as 17-hydroxy-11-dehydrocorticosterone [212]. In a study of various compounds for their action against toxic material (typhoid vaccine) it has been found that the relative potencies of several preparations in units per milligram (protection unit) are as follows: 11-dehydro-17-hydroxycorticosterone, 5.5; 11-dehydrocorticosterone acetate (natural and synthetic), 3.6; 11-dehydrocorticosterone, 3.1; corticosterone benzoate, 1.3; 11-ketoprogesterone, between 0.5 and 3.0; desoxycorticosterone, 0.5; progesterone, less than 0.3; and desoxycorticosterone acetate, acetoxypregnenolone and methyl androstenediol, all less than 0.1. It is clear from the above that all the compounds possessing an oxygen atom at C-11 can give considerable protection to animals from typhoid vaccine. The protective capacity of any given substance has been found to be closely parallel to its activity as determined by the muscle-work test of Ingle. It seems likely, therefore, that a direct relationship may exist between the ability of these substances to protect against typhoid vaccine and their rôle in the regulation of carbohydrate metabolism [213].

#### PHYSIOLOGY OF THE ADRENAL CORTEX

The adrenal cortex is indispensable for life, as shown by bilateral adrenalectomy. Its main life-sustaining functions are concerned with electrolyte and water metabolism.



On the basis of observations in Cushing's Syndrome, Albright and Brown have suggested that the adrenal cortex produces at least two hormones, the "S" (sugar) hormone, concerned with glyconeogenesis from protein, and the "N" (nitrogen) hormone, concerned with the retention of body nitrogen and the synthesis of new protein. Loeb classifies cortical activity as primary and secondary. The primary activity, essential for the maintenance of life, is concerned with electrolyte and water metabolism, and is identical with that of desoxycorticosterone. The secondary activity is called upon in emergency and serves for protection against various forms of stress. According to Goldzieher [19], the emergency function is catalytic in nature, facilitating the utilization of oxygen by the tissues. Any interference with oxygen utilization in the tissues calls immediately for an increased output of cortical hormone and produces a compensatory hyperplasia of the cortex.

High altitudes demand an increased function of the adrenal cortex to which response is made by compensatory hypertrophy. Prolonged anoxia, in animals kept in artificially reduced atmospheric pressure, causes degenerative changes of the cortex, with eventual exhaustion of cortical function [20]. The adrenal cortex of the foetus is disproportionately large because its arterial blood is only 63 per cent saturated with oxygen as compared with a saturation of 95 per cent of the maternal blood. The oxygen tension of the foetal blood is only 14 mm. as compared with a tension of 100 mm. in the alveoli of the breathing lung [21]. Extreme enlargement of the adrenal cortex was found in four cases of erythroblastosis foetalis [5].

Anoxia causes not only an increased demand on the adrenal cortex but also a discharge of adrenalin and sympathin. Estrada and others [22, 23] found a decline in the respiratory quotient of the brain even in the presence of glucose. Anoxia increases the permeability of membranes and passive congestion, whether due to cardiac failure or obstacles in the peripheral circulation, produces oedema probably due to lack of cortical hormone. Bryan and Ricketts [24], in five experiments on four human subjects, found, with the possible exception of a slight increase in potassium excretion in two subjects and a transient rise in 17-ketosteroid excretion in one, no evidence that the human adrenal cortex is influenced by chronic intermittent anoxia.

In summary it can be concluded that there are three main types of adrenal cortical function: (1) electrolyte metabolism, (2) protein and carbohydrate metabolism and (3) androgenic production. They may vary independently of one another both qualitatively and quantitatively, thus leading to a variety of metabolic conditions and clinical

syndromes; for example, in Cushing's syndrome the glycogenic corticoids may be greatly increased with normal or slightly increased 17-ketosteroids; conversely in hirsutism the 17-ketosteroids may be increased and the glycogenic corticoids remain within normal limits [214].

### **Adrenal Cortical Insufficiency**

Atrophy of the adrenals is found in senescence as well as in cachexia. Lack of vitamins, especially of nicotinic acid, causes atrophy of the cortex. Chronic pulmonary tuberculosis, hypothyroidism and Simmonds's disease are associated with atrophic changes of the cortex, which are usually accompanied by the symptoms first described by Addison.

In certain patients with neoplasm not arising in a gonad or adrenal cortex, 17-ketosteroid excretion is unusually low [216]. In the pregnant rat adrenalectomy produces a normal number of offspring with significantly subnormal weight. The mortality rate is high, owing to diminution of lactation in the mother, in cases in which the adrenalectomy was recent. The average weight of the suprarenal glands in the offspring of the adrenalectomized rats is increased [217].

Cortical atrophy can be produced with progestogen or testosterone if large doses are used. Unilateral atrophy (termed by Selye "compensatory atrophy") is produced by a contralateral hyper-secretory cortical tumour [215].

### **Adrenal Cortical Hyperfunction**

Any stimulus to the "alarm reaction" will elicit cortical hypertrophy. Compensatory hypertrophy of the cortex occurs owing to structural injury to one of the adrenal glands. Hormonal stimuli, like thyroxine [25, 26] and oestrogen [27], may produce hypertrophy of the cortex. Prolonged doses, however, produce degenerative changes. Cold, altitude and reduced atmospheric oxygen cause adrenal and cortical hypertrophy.

Diffuse hypertrophy of the cortex is also found in adult women with the virilism syndrome. Parhon and Zurgravu [28] have found the adrenals to be of larger size in certain types of insanity, for example, in schizophrenia.

Normal excretion of 17-ketosteroid is 13 mg. per day for females and 18 mg. per day for males. The difference of 5 mg. per day may represent the testicular contribution. In both sexes the normal physiological range of excretion is very wide. In normal man ranges from 9 mg. to 28 mg. per day have been found. In normal women



the range is even wider, since values of from 4 mg. to 23 mg. per day have been obtained in apparently normal individuals. Carcinoma of the adrenal cortex of females of all ages is almost always associated with high excretions of 17-ketosteroids.

Biological assays show that these substances are excreted in increased amounts after operations, burns, or other tissue damage. These findings appear to substantiate the view that the adrenal cortex plays a part in the reaction of the organism to such stresses.

Adrenal cortical hyperplasia does not appear to increase the corticosteroid excretion, but it does increase that of 17-ketosteroid. On the other hand, in Cushing's syndrome the corticosteroid output is very greatly augmented while that of 17-ketosteroid is raised but little. According to Scowen and Warren it appears possible that the corticosteroid and 17-ketosteroid production (or at least production of 17-ketosteroid precursors) are not necessarily linked. Furthermore, it is possible that there are two distinct adrenocorticotrophic hormones elaborated by the anterior pituitary, one of which stimulates corticosteroid production and the other androgen production in the adrenal cortex. The androgenic stimulation that can occur in acromegaly and the increased corticosteroid production in basophilism are very interesting in this connection [218].

In pregnancy there is an initial increase in adrenal cortical function, as shown by a rise in excretion of corticoids in the first trimester which usually returns to normal levels by the 100th-120th day. Between the 140th and 160th days the urinary corticoids increase again, reaching relatively high values. A falling off in excretion usually occurs in the last month, and shortly after parturition the values are back to normal again [59, 219].

Experimentally, hypertrophy of both cortex and medulla can be produced by prolonged muscular exertion, alcohol poisoning, administration of sulphur and injection of insulin.

### **Electrolyte and Water Metabolism**

This function of the adrenal cortex is very complex in nature. Adrenal insufficiency causes, together with a loss of water, a loss of sodium and chloride and an increase of potassium in the plasma. Through osmotic changes in the cells, potassium migrates from the cells into the extra-cellular spaces, and the interstitial fluid passes into the cells. The carbon dioxide capacity of the blood is decreased while the oxygen-combining power is increased. The utilization of oxygen in the cells is impaired, however.

The rise in the potassium level, being caused by leakage from the



issue cells into extra-cellular fluids, may also be due to reduced excretion through the kidneys. A low plasma potassium, on the other hand, may be due to prolonged deprivation or to increased renal elimination of potassium. Depletion of cellular potassium can be produced experimentally by the administration of desoxycorticosterone in excessive doses, and is also observed in hyperinterrenal diseases, including Cushing's Syndrome [29].

According to Goldzieher [30], the increase in blood potassium is not quite as specific a sign of cortical deficiency as was previously assumed, for the rise is prevented not only by the cortical hormone but also by maintenance of a normal plasma level. In diabetes insipidus, cortical extract fails to increase potassium excretion unless potassium is supplied [31].

Desoxycorticosterone acetate decreases the concentration of potassium and increases the concentration of sodium and chloride [32], producing increased diuresis not accompanied by renal hypertrophy [33].

Corey and Britton [34] found a distinct antagonism between desoxycorticosterone acetate and the posterior pituitary hormone. Large doses of desoxycorticosterone acetate, in fact, produce a syndrome similar to diabetes insipidus.

Adrenal cortical deficiency causes, in addition to osmotic changes in the cell membranes, increased permeability of the capillaries, thus permitting a shift of fluid from the blood to the tissues [35]. Menkin [36] proved by injecting a blue dye into the circulation that deficiency of the adrenal cortex was responsible for the increased capillary permeability. The exposure of the abdomen of rabbits to the carbon arc lamp for 30-40 minutes at a distance of 1 metre, followed by the intravenous injection of the dye trypan blue, produced an extreme blue coloration of the skin and viscera, many times more marked than that of the controls. Intramuscular injection of 10 mg. of desoxycorticosterone acetate, or of 5 c.c. of cortical extract, at the time of irradiation, or before, prevented the increased permeability of the minute vessels, caused by the action of substances released from tissue undergoing inflammatory changes [37].

Adrenal cortical extracts have been shown to reduce the rate of oedema formation in perfused frogs' legs. The effectiveness of cortical hormone in reducing the rate of oedema formation does not always correspond with the intensity of other cortical functions [38]. Desoxycorticosterone acetate does not protect the capillaries or membranes and it may even increase their permeability if given in sufficient concentration. For example, in the

pre-menstrual female, desoxycorticosterone acetate produces marked oedema [39].

### Carbohydrate Metabolism

Pancreatic diabetes of the experimental animal is alleviated by adrenalectomy and exacerbated by injection or feeding with cortical extracts. The antagonism of the adrenal cortex to insulin is further demonstrated by the fact that administration of cortical extract prevents insulin hypoglycaemia. Diabetes in rats, produced by alloxan, showed a marked reduction or complete disappearance after adrenalectomy [40].

Dosne [41] showed that experimental diabetes produced in the toad by subtotal pancreatectomy could be prevented by the simultaneous removal of the anterior lobe of the hypophysis; subsequent injections of anterior pituitary tissue produced hyperglycaemia. Adrenalectomy attenuated the pancreatic diabetes, which reappeared however on injection of anterior pituitary extract. Extirpation of the thyroid in no way influenced the production of pancreatic diabetes. A case of diabetes mellitus associated with a large tumour of the right adrenal cortex, has been reported recently with cure following the removal of the tumour [42].

The gluconeogenesis produced by the adrenal cortex occurs primarily at the expense of proteins. An excess of cortical extracts combined with adequate intake of carbohydrate decreases the rate of carbohydrate oxidation and causes increased accumulation of glycogen in the liver and muscles. The breakdown of glycogen in the liver is prevented by an excess of cortical extracts, the carbohydrate supplied to the tissues deriving in this case from protein and fat with resulting hypoglycaemia (Gierke's disease).

Insulin has a stimulating action on the adrenal cortex. In rabbits [43], mice [44] and pigeons [25, 46], adrenal hypertrophy has been reported after prolonged administration of insulin.

### Protein Metabolism

Excess of cortical hormone accelerates, just as its lack retards, protein catabolism in the tissues. Almost all stimuli which increase cortical function or cortical hyperplasia (e.g. increased thyroid activity, cold, toxins, infections, burns, shock, pancreatic diabetes and anoxia) are accompanied by a breakdown of protein. Histamine, peptone or other biogenic amino-acids stimulate the adrenal cortex.

A high protein diet considerably increases the weight of the cortex. Tepperman and others [47] found that such a diet produced hyper-



rophy of the suprarenals in the albino rat. Enucleation of the gland showed that the enlargement was primarily cortical.

### **Fat Metabolism**

Adrenalectomy causes extensive loss of weight and complete disappearance of the subcutaneous fat depots. The adrenalectomized animal suffering from inadequate absorption of fat from the intestines can be restored to normal by the administration of cortical extract [50]. The cortical hormone is also indispensable for transport and storage as well as mobilization of fat from the depots [50]. Increased demand for cortical function is associated with cholesterolaemia.

In laboratory animals, adrenal cortical insufficiency is characterized by a slower than normal rate of absorption of fat, a decrease in the amount of fat transported to and deposited in the liver (under conditions which normally cause fatty livers), a smaller than normal response to ketogenic agents, and a normal or high respiratory quotient [51].

### **Basal Metabolism**

Deficiency or extirpation of the cortex may cause lowering of the basal metabolism and heat production [52]. Administration of cortical hormone restores the basal metabolic rate to normal but fails to increase the basal rate in the intact animal. According to Marine and others [53, 54] adrenalectomy causes a rise in the basal metabolic rate of experimental animals, and administration of cortical hormone diminishes the oxygen consumption in normal and thyrotoxic subjects. In Croster's experience, all cases after unilateral adrenalectomy passed into a state resembling acute hyperthyroidism, with a falling blood pressure and a rising pulse rate. Lugol's solution was extremely helpful in these cases [205].

On the whole, the effect of adrenal cortical hyperfunction or deficiency on basal metabolism depends very much on the activity of the other glands, particularly the thyroid, and on the duration of the disorder.

### **Haemopoietic System**

Daily injections of adrenotrophic hormone into mice produce an increase in haemoglobin and in red cells, an absolute lymphopenia, and an increase in polymorphonuclear leucocytes. Adrenalectomy in rats and mice produces a trend towards anaemia with absolute lymphocytosis and a depressed polymorphonuclear production [55].



## Growth

Pituitary growth hormone is antagonistic to the adrenal cortex.

Compound "E" suppresses growth in growing animals, probably by increasing protein breakdown, whereas the growth hormone increases protein anabolism [56] as shown by nitrogen retention.

## Gonadal System

There is no doubt that a relationship exists between sex and the adrenal cortex. Extraction of the cortex yields a number of sterols, e.g. progesterone, oestrone and various androgens, as well as substances with lactogenic and gonadotrophic activity. A special part of the cortex, "Zone X", seems to be responsible for the androgenic activity, the protoplasm of its cells giving a fuchsinophil reaction.

Chronic adrenal insufficiency is associated with ovarian atrophy, amenorrhoea and sterility, and in the male with loss of libido and impotence. Hyperfunction of the cortex, due to hyperplasia or tumour, has a virilizing effect in the female and causes the premature appearance of secondary sexual characters in the male.

In the normal mechanism of sexual development, the adrenal cortex plays the part of a bisexual accessory sex gland which is active throughout life and secretes both androgenic and oestrogenic hormones under the control of the pituitary [12]. In women the occurrence, during early foetal life, of a short period of androgenic and heterosexual development of cortical origin introduces an element of heterosexual instability, tending to intersexuality under conditions of adreno-pituitary imbalance. The greater stability of the male, in whom no comparable oestrogenic period occurs, results in the much greater rarity of heterosexual changes in that sex.

Pseudo-hermaphroditism and adolescent virilism are examples of adreno-pituitary imbalance in the female leading to intersexuality. It is doubtful whether strictly comparable syndromes occur in the male. The common type of pseudo-hermaphroditism is a sex-inversion of the female due to a cortical androgenic hyperfunction occurring before the end of the fifth month of foetal life. Adolescent virilism is the result of a similar cortical hyperfunction occurring after that date but probably originating during foetal life.

Hypertrophy or tumours of the adrenal cortex may manifest themselves by three different syndromes:

1. Cushing's syndrome, showing evidence of an increase in the glycogenic corticoids and mild diabetes due to hypergluconeogenesis,

which is often insulin resistant sometimes with increased nitrogen excretion.

2. Adrenogenital syndrome, characterized by the androgenic activity of the adrenal cortex and by the production of very little of the so-called metabolism-regulating hormones of the cortex. These cases show absence of diabetes and the presence of sexual and somatic precocity.

3. Feminization in the male with the salient features of gynaecomastia and decreased libido. The Friedman test is usually positive [220].

Pituitary basophilism is an example of adreno-pituitary imbalance in which the pituitary is most probably primarily at fault, the adrenal cortex being involved secondarily. The syndrome may show either isosexual or heterosexual changes according to whether the androgenic or oestrogenic element of cortical activity is the more stimulated by pituitary hyper-secretion. Symptoms of intersexuality in women are the more common, indicating again the greater instability of the female sexual make-up, though feminization of the male may occur.

Cortical carcinoma of the adrenal may cause a direct disturbance of the normal adreno-pituitary sexual mechanism, due to the uncontrolled production, by the tumour cells, of either androgenic or oestrogenic secretions, and leading to isosexual or to heterosexual changes. The adreno-pituitary relationship is again seen in certain cases of cortical carcinoma, in which the symptoms are largely those of pituitary basophilism and are in fact associated with histological changes in the basophil cells of the pituitary.

Unusually large amounts of androgenic hormone in the urine have been found in cases of adrenal virilism. The removal of one adrenal gland caused immediate reduction in the male hormone excreted [12]. The remaining adrenal contained no androgen at all or only very little [7, 58, 12]. Broster and Vines [11] demonstrated that in sections of suprarenal glands removed at operation, and stained with fuchsin and counterstained with aniline blue, the protoplasm of the cortical cells were bright red in virilism (fuchsin), in contrast to the cortical cells of normal people which stained blue. It was later found that virilism involved both adrenals, not only the gland removed on operation. This fuchsinophil reaction was also present in the acidophil cells of the pituitary, the interstitial cells of the testes, the young corpus luteum cells, and in scattered cells in the stroma of the ovary after the menopause [59].

In examining the adrenals of 60 foetuses, Vines demonstrated that



this same staining reaction was present in the cortical cells of embryos of both sexes. In the male it was strong between the ninth and seventeenth week, but in the female it appeared about the eleventh week, reaching maximal intensity about the fourteenth week and then rapidly decreasing just about the time when the basophil cells of the pituitary gland were beginning to appear [59, 12]. During further investigations, Broster showed that adrenal virilism is not necessarily dependent on cortical hypertrophy, but may occur in the presence of normal-sized glands. In one particular case, in which the gland was found on removal to be normal in size and weight, an intense fuchsinophil reaction was observed.

Investigating intensity of staining Broster and Vines [11] found that the same degree of virilism may be associated with a normal-sized gland staining a strong red as with a hyperplastic gland staining only moderately.

Excessive amounts of oestrogen were noted in some cases of virilism and Cushing's syndrome, which accounts for the feminism sometimes associated with adrenal cortical disorders in the male [60, 61]. Injections of adrenotrophic extracts cause enlargement of seminal vesicles and prostate in castrated rats, but only in the presence of the adrenal cortex [62]. According to Ehrenstein and Britton [63], the cortex contains a factor which acts as activator of ordinary androgens.

The androgenic activity of the adrenal cortex is estimated by quantitative determinations of the urinary androgens or 17-ketosteroids. Definite knowledge is still lacking as to the mother substance of the urinary 17-ketosteroids. Marker suggested that it was Kendal's compound E; Cuyler and others [64] found that it was neither desoxycorticosterone nor its esters (see Chapters VIII, IX).

There is some evidence that the adrenal cortex is necessary for the maintenance of normal lactation. Whether it secretes a lactogenic hormone or merely augments the action of the pituitary hormone is not established.

### Protective Effects

The adrenal cortex protects against all kinds of noxious influences as, for instance, cold, loss of blood, poisons, infections and anaphylactic shock. In addition, it prevents inactivation of its own hormone as well as of the medullary hormone. At the same time, it sensitizes the peripheral receptor tissues for the response to adrenalin.

Cortical deficiency causes a marked depression of antibody formation [65, 66, 67]. The amount of antigen that will produce maximal antibody titres in the adrenalectomized animal is many times that



required in normal animals [68]. According to Fox and Whitehead [69] treatment with cortical extract is invariably followed by increase in haemolysins. Steinbach [70] found that tuberculosis occurred in 100 per cent of adrenalectomized animals and only in 50 per cent of controls, both exposed to infection. The administration of adrenal cortical extract protects guinea pigs against diphtheria toxins [71] and *Clostridium welchii* infections [72], and rats against toxic effects of typhoid vaccine [213]. In the human subject infection and trauma elicit a rise in the urinary excretion of cortin [221].

Adrenal enlargement accompanied by depletion of lipid or reversal of lipid pattern was found associated with inflammatory disorders, cachexia, pemphigus and protracted emesis [5]. Administration of cortical extract has proved effective in various infectious diseases. Particularly favourable results have been obtained by Goldberger in cases of upper respiratory infections, bronchial pneumonia, influenza and post influenzal asthenia. Such asthenia is prevented or rapidly improved by adequate dosage of cortical extracts. The effects of the adrenal cortex in this respect depend largely on activation of the reticuloendothelial system or indirectly on the maintenance of normal metabolic conditions [73].

The greater susceptibility of the adrenalectomized animals to anaphylaxis is due to their generally increased sensitivity to endogenous toxic substances such as histamine. Selye [74, 75, 76] demonstrated that the resistance of rats and dogs to shock is increased by cortical extract or corticosterone. Desoxycorticosterone acetate proved effective against shock [138]. Swingle and co-workers [77], however showed, that desoxycorticosterone acetate would protect the normal as well as the adrenalectomized animal against shock, if this was due only to disturbances of permeability, loss of fluid and peripheral circulatory collapse. If, however, further damage was inflicted upon the experimental animal, only full cortical extract or corticosterone proved effective. Perla and Marmorston [68] have also shown the value of desoxycorticosterone acetate in restoring normal capillary permeability in shock.

The protection of the cortex by its own hormone takes place through the storage of various substances such as lipoids, vitamins and sulphur-containing compounds [78]. In cortical hyperfunction, the lipid material is present in great abundance, whereas muscular activity, as well as other influences which increase the demand for cortical function, decrease the cortical lipoids. Their disappearance in fatal acute infections represents morphological evidence of adrenal insufficiency.

Another substance stored in the adrenal cortex is choline, with its derivative, acetylcholine. Acetylcholine is vasotonic and its relationship to adrenalin is one of antagonism. Antagonism has not been observed, however, in relation to carbohydrate metabolism. The most important effect of acetylcholine is the stimulation of the adrenal medulla and the subsequent discharge of adrenalin, especially in emergency. By this mechanism acetylcholine in large doses causes hypertension; whereas small doses lower the blood-pressure [79, 80]. Acetylcholine inhibits the relaxation of the intestinal musculature, causing its contraction; and of the bronchial musculature, causing asthmatic attacks in susceptible persons.

Glutathione, also found in the adrenal cortex, is a sulphur-containing substance of unknown chemical nature. The sulphur is present in unusual amounts, suggesting that its storage is another function of the adrenal cortex. Reiss and Winter [81] observed reduction of blood-sulphur after injections of cortical extracts. According to Goldzieher [82], the adrenal cortex takes up the sulphur from the blood in order to transform it into active substances, among which glutathione is the most important. Its physiological significance is still little known.

Administered to diabetic patients in small doses, sulphur decreases the hyperglycaemia and glycosuria; whereas in larger doses it produces cortical hyperplasia and increased hyperglycaemia and glycosuria.

The protective rôle of the suprarenals in the general adaptation syndrome and the disease of adaptation recently described in detail by Selye [222] is summarized in its main points.

Adaptation to surroundings is one of the most important physiological reactions in life; one might even go so far as to say that the capacity of adjustment to external stimuli is the most characteristic feature of living matter [223].

1. The general adaptation syndrome is the sum of all non-specific systemic reactions of the body which ensue upon long-continued exposure to stress.

2. The alarm reaction is the sum of all non-specific systemic phenomena elicited by sudden exposure to stimuli to which the organism is quantitatively or qualitatively not adapted. The alarm reaction may in turn be subdivided into two more or less distinct phases: the phase of shock and the phase of counter-shock.

3. The stage of resistance represents the sum of all non-specific systemic reactions elicited by prolonged exposure to stimuli to which



the organism has acquired adaptation as a result of continuous exposure.

4. Finally, the stage of exhaustion represents the sum of all non-specific systemic reactions which ultimately develop as the result of very prolonged exposure to stimuli to which adaptation had been developed, but could no longer be maintained.

One of the most important morphological changes in the alarm reaction is the enlargement of the adrenal cortex. Its individual cells hypertrophy and discharge their lipid granules. This has been considered a sign of increased endocrine activity. True hyperplasia is usually less pronounced. These changes take several hours to develop and, as a rule, do not reach their peak until counter-shock phenomena are evident [224]. The adrenal changes, and especially the rather characteristic lipid distribution pattern in the cortex during the three stages of the adaptation syndrome, have repeatedly been described [4, 225-7]. The cholesterol content of the adrenal cortex rapidly decreases under the influence of alarming stimuli [228, 229]. It appears that the cortical hypertrophy and lipid loss of the alarm reaction subside quite regularly during the stage of resistance but reappear during the stage of exhaustion.

The chromaffin granules of the medulla are discharged within a few minutes after exposure to an "alarming stimulus", and the medullary cells become vacuolized. The adrenals play an important part during the alarm reaction and secrete adrenalin immediately after exposure, even before the shock phase is evident, while corticoid secretion is a counter-shock phenomenon.

The thymus also shows very conspicuous changes during the alarm reaction, inasmuch as it undergoes acute "accidental involution" which becomes most pronounced during the counter-shock phase when the adrenal cortex reaches its maximum development. This involution is inhibited by adrenalectomy or hypophysectomy [224, 230]. The lymph nodes, the spleen and other lymphatic organs are almost as markedly affected as the thymus, although their involution is not quite as rapid and is not as completely prevented by adrenalectomy.

The hypophysis probably occupies the most important central position in the constructive or defense reactions which are essential for resistance and adaptation to non-specific damage. The anterior lobe often shows degranulation, especially of the eosinophils, and sometimes marked waves of nuclear pyknosis. The borderline between anterior and posterior lobe may become rather irregular with



signs of "basophilic invasion" [224] but these changes are inconstant. There is good evidence to show that under such influences the hypophysis produces increased amounts of corticotrophic hormones, for in hypophysectomized animals the adrenal cortex shows no signs of hypertrophy or increased functional activity. It has been claimed that during the alarm reaction an excess of corticotrophic hormone can actually be demonstrated in the blood [231]; but because of the unusual bio-assay method employed, this work requires confirmation.

There can hardly be any doubt that at least during, and immediately after, the alarm reaction stage, the enlargement of the adrenal is accompanied by a pronounced increase in corticoid hormone production. It has been found that following operations, burns and other types of exposure to alarming stimuli, the corticoid activity of the urine rises considerably above normal [232-7]. It has also been claimed that the increased diuresis noted in aviators at high altitude may be the result of increased cortical hormone secretion, since the corticoids are known to have diuretic properties. It should be pointed out, however, that this interpretation has not been proven as yet.

The chromaffin system has been shown to play a prominent rôle only during the first few minutes of the alarm reaction when the blood pressure and the blood sugar show their transitory rise.

Recently, several interesting facts have come to light which indicate that the alarm reaction may also have an important influence upon serological immune reactions. It has been found that lymphocytes of normal rabbits contain a globulin identical with the normal serum  $\gamma$ -globulin which is important for antibody formation [238]. Furthermore, "labelled globulin" (antibody protein) has been demonstrated in lymphocytes isolated from the lymphoid tissue of immunized mice [239]. The presence of immune bodies in lymphocytes has also been confirmed in the immunized rabbit [240]. All these observations suggest that the lymphatic organs play an important part in immune reactions. It has been noted, furthermore, that corticotrophic hormone secretion accelerates the rate of release of antibodies from the lymphoid tissue of immunized rabbits [241] and that alarming stimuli enhance the antibody titre in the sera of previously immunized animals [242]. Finally, it is claimed that the enhancement of antibody titre, produced by alarming stimuli, can be elicited by corticotrophic pituitary extracts and adrenal cortical extracts, but not by desoxycorticosterone acetate. Cortical extracts, but not corticotrophic extracts, are effective in this respect, even in the adrenalectomized animals. From these experiments it has been concluded that "adrenal

cortical medication is essential for the control of the release of antibody from lymphocytes" [243].

### Corticotrophic Factors

The function of adrenal cortex depends on a great number of stimuli in addition to the corticotrophic hormone of the anterior pituitary (see Chapter I). The adrenal cortex is relatively independent of the pituitary gland, however, as shown, for example, by its continued function under the influence of insulin, thyroid and small doses of oestrogen after hypophysectomy, and also by the fact that the hypophysectomized animal does not show evidence of total adrenal insufficiency. According to Goldzieher [19], adrenal cortical insufficiency in experimental animals and humans mainly affects carbohydrate electrolyte and protein metabolism, whereas only slight disturbances in vascular permeability and electrolyte metabolism occur in the hypophysectomized animal and in the advanced stages of Simmonds's disease.

The corticotrophic factor of the anterior lobe is, however, a prerequisite for the response of the adrenal cortex to almost all stimuli. The effect of the pituitary corticotrophic hormone is markedly augmented by addition of a dilute suspension of pituitary tissue [84], similar to the synergic effect of the anterior lobe extract upon chorionic gonadotrophin. Stimuli which may cause adrenal cortical enlargement are the following:

(1) Oxygen deficiency, either absolute, or caused by other factors such as thiamine deficiency, exposure to cold and physical exercise.

(2) Factors causing increased breakdown of proteins, injections of peptone [85], feeding of proteins [86, 87], inanition, and toxic substances (including alkaloids, heavy metals, phenol derivatives, formaldehyde, ammonia and bacterial toxins).

(3) Hormonal Factors.

*Oestrogens*.—Injections of oestrogen in moderate doses cause marked hypertrophy (though not in the hypophysectomized rat [167], while excessive doses produce cortical atrophy. In the hypophysectomized rat, the size of the cortex can be maintained with oestrogen [48].

*Insulin*.—Prolonged administration of insulin produces adrenal hypertrophy (even in the hypophysectomized pigeon).

*Castration*.—Castration of the male animal is followed by adrenal hypertrophy [89]. This is inhibited by the male sex hormone.

*Thyroid*.—The thyroid hormone is one of the most active stimulants of the adrenal cortex [88, 90, 91]. Enlargement of the cortex

follows administration of the hormone, whereas thyroidectomy produces atrophy of the cortex and inhibits its response even to the injection of pituitary extracts [92]. Prolonged stimulation by the thyroid produces first hyperplasia and ultimately exhaustion of the adrenal cortex [93].

#### DESOXYCORTICOSTERONE ACETATE

This synthetic crystalline preparation has a very powerful sodium- and chloride-retaining effect but lacks the carbohydrate-regulating factor.

The adrenal steroid corticosterone, with oxygen atoms in the C<sub>3</sub>, C<sub>11</sub>, C<sub>20</sub> and C<sub>21</sub> positions, is effective in correcting both mineral and carbohydrate disturbances in metabolism. The removal of the oxygen atom from position C<sub>11</sub> (11-desoxycorticosterone acetate) deprives the compound of its carbohydrate-regulating activity but enhances sodium- and chloride-retention. The addition of an oxygen atom at C<sub>17</sub> (17-hydroxycorticosterone) increases carbohydrate-regulating potency and decreases sodium- and chloride-retention, or it may even induce sodium- and chloride-loss in some circumstances [94].

Desoxycorticosterone acetate decreases the concentration of potassium and increases the concentration of sodium and chloride. It has apparently no effect on muscular efficiency [95]. It does not protect the capillaries or membranes and, if given in sufficient concentration, may even increase their permeability. In the pre-menstrual female, it produces marked oedema [39].

Corey and Britton [34] found a distinct antagonism between desoxycorticosterone acetate and the posterior pituitary hormone. Thus, it may produce increased diuresis, and in fact large doses produce a syndrome similar to diabetes insipidus.

Administration of desoxycorticosterone acetate to rats, 2 mg. daily for 1 month, resulted in adrenal atrophy in the male but not in the female. After hypophysectomy administration of desoxycorticosterone acetate produced further atrophy, and the hypothesis has been advanced that adrenal atrophy following desoxycorticosterone acetate is not mediated through the pituitary [96]. Desoxycorticosterone has an oestrogenic effect, as shown by the induction of gynaecomastia during the treatment of an Addisonian male patient. When treatment was stopped, the mammary enlargement disappeared in spite of continuous large doses of whole cortical extract [97]. There is some evidence that desoxycorticosterone has a lactation inhibiting effect [98].



Hoffman and others [99] observed that desoxycorticosterone acetate administered to adult rabbits was excreted as pregnanediol, and that the amount excreted was the same as followed the injection of an equivalent amount of progesterone. It is suggested that pregnanediol of adrenal origin may arise from desoxycorticosterone as well as from progesterone.

Experiments performed by Courrier [244] on rabbits proved that desoxycorticosterone can prevent abortion in the absence of a corpus luteum and, on the other hand, that it may bring about interruption of gestation. When 20–30 mg. of desoxycorticosterone acetate were given daily to castrated pregnant rabbits, pregnancy was maintained in the absence of the ovaries, the foetuses grew regularly, and their number was normal. It required about 5 mg. of progesterone daily to produce the same result.

When 20 mg. of desoxycorticosterone acetate was administered daily for 6 days to pregnant rabbits with intact ovaries and the animals were examined 12 days later, it was found that the gestation had been interrupted. In these cases the desoxycorticosterone either affected the corpus luteum directly or indirectly through the pituitary gland.

It was shown in castrated female guinea pigs treated with fibromatogenic quantities of diethylstilboestrol that the addition of desoxycorticosterone acetate prevented oestrogen-induced abdominal fibroids. The average fibrous tumoral effect diminished by the action of desoxycorticosterone from 5.5 to 1.7 units. Concentration of chloride, sodium and potassium in the blood of the groups treated with diethylstilboestrol, desoxycorticosterone or both was within the same range as in animals not treated. These results show that the anti-fibromatogenic quantity of desoxycorticosterone acetate is smaller than those doses which might interfere with the concentration of chloride, sodium or potassium in the blood [245].

### **Desoxycorticosterone Overdosage**

Selye and others [100] showed, in the rat, that overdosage of desoxycorticosterone acetate elicits polyarthritis which morphologically resembles that seen in acute rheumatic fever. Joint lesions are more readily produced by desoxycorticosterone acetate in adrenalectomized or thyroidectomized than in intact rats, especially if they are exposed to cold. The authors interpret the similarity between the experimental lesions produced by desoxycorticosterone acetate and the manifestations of rheumatic fever as an indication that the adrenal cortex may play an important part in the pathogenesis of rheumatic and rheumatoid conditions in man.

Overdosage of desoxycorticosterone acetate produced cardiac lesions (necrosis of myocardial fibres and replacement by fibroblasts) in rats fed on a diet low in potassium and high in sodium; but these changes could be prevented by the addition of potassium chloride to the drinking water [101].

Selye [102] produced signs of fluid retention ascites, pericardial effusion and nephrosclerosis in white Leghorn chicks injected daily with 0.5 mg. desoxycorticosterone acetate subcutaneously. Using the pellet implantation technique, he also showed that in sensitized rats less than 1 mg. of desoxycorticosterone acetate per day suffices to produce severe periarteritis nodosa of the cerebral vessels and malignant nephrosclerosis [246].

#### SUMMARY OF EFFECTS PRODUCED BY ADRENAL CORTICAL DEFICIENCY AND BY ADRENALECTOMY

Loss of sodium and chlorides, with increased potassium in the plasma.

Shift of potassium from the cells into the intercellular spaces and accumulation of sodium and water in the cells.

Reduced intestinal absorption of carbohydrates with a decrease in the blood-sugar level.

Delay in production and removal of lactic acid, so that the lactic acid in the blood is either decreased or increased.

Reduction of gluconeogenesis from proteins.

Retardation of protein catabolism.

Diminution of specific dynamic action of proteins.

Reduction of intestinal absorption of fat.

Marked increase of the respiratory volume and respiratory quotient, and a decrease of the alveolar carbon dioxide.

Decrease of the blood carbon dioxide capacity, and change of the blood pH to the acid side; increased oxygen combining power of the blood and decreased oxygen utilization by the tissues.

Anaemia with increase in polymorphonuclear leucocytes. Hyperplasia of the lymphatic tissue involving lymph nodes as well as lymphatic tissue of the intestines.

Increase of blood-urea and non-protein nitrogen.

Increased permeability of the capillaries.

Inability to control body temperature.

Loss of weight.

Decreased resistance to various infections and poisons, including narcotics, diphtheria toxin, typhoid vaccine and histamine.

Pathological changes in other organs, e.g. gastric ulcers, atrophy of the gonads, regeneration and increase in the size of the thymus, and increased thyroid activity.

Decrease in blood cholesterol.

#### SUMMARY OF EFFECTS PRODUCED BY ADRENAL CORTICAL HYPERFUNCTION AND BY ADMINISTRATION OF CORTICAL EXTRACTS

Sodium and chloride retention with increased excretion of potassium. Shift of potassium from the intercellular spaces into the cells and accumulation of sodium and water in the intercellular spaces.

Increased intestinal absorption of carbohydrates, with increase in the blood-sugar level.

Increased production and removal of lactic acid.

Increased gluconeogenesis from proteins and inhibition of glycolysis.

Acceleration of protein catabolism.

Increased specific dynamic action of protein.

Increased intestinal absorption and deposition of fat.

Decrease of the respiratory volume and respiratory quotient, and increase of the alveolar carbon dioxide.

Increase of the blood carbon dioxide capacity with decreased oxygen combining power of the blood and increased utilization of oxygen by the tissues.

Increase in haemoglobin and red cells.

Acceleration of thymic involution and prevention of hyperplasia of the lymphatic tissue.

Decrease of blood-urea and non-protein nitrogen.

Decreased permeability of the capillaries.

Control of body temperature.

Increase of body weight.

Increase of resistance to various infections.

Decrease of thyroid activity.

Increase of blood cholesterol.

#### ADRENAL MEDULLA

The medulla is situated in the middle of the suprarenal gland and surrounded by the adrenal cortex. There is no sharp line of demarcation between these two portions of the gland.

The cells of the medulla show a peculiar affinity to bichromates which stain the cytoplasm yellowish brown; they have accordingly



been called chromaffin or pheochrom cells. Affinity to bichromates disappears within 12 hours after death. The chromaffin reaction of the medulla is not uniform, and shows light patches which characterize the pheochrom cells in the non-secretory phase, for only part of the total number of medullary cells is actively secreting at any given moment.

#### PHYSIOLOGY

Abel proposed the name "Epinephrine" for the vasoconstricting principle isolated from the adrenal medulla; but the original trade name "Adrenalin" is in general use. Stolz and Dakin were the first to synthesize this substance. Phenylalanine is the most likely precursor of adrenalin [103]. Arnow [104] converted phenylalanine into dehydroxyphenylalanine *in vitro* by ultra-violet radiation. At the same time melanine is formed, which can be produced *in vitro* by dihydroxyphenylalanine.

Adrenalin is inactivated by enzymes such as tyrosinase and catechol oxidase. Heirman [105] has shown that catechol oxidase transforms adrenalin into adrenoxine, a vaso-dilator substance, provided that the concentration of adrenalin is not too high. The action of adrenalin on the uterus, intestines and other organs is explained by their catechol oxidase [106]. The most important site for the inactivation of adrenalin is the liver, presumably owing to a special enzyme which is inhibited by narcotics.

The effects of adrenalin on the various tissues are identical with those obtained by stimulation of the sympathetic nervous system. The lower thoracic sympathetic chains discharge an adrenalin-like substance demonstrable by direct stimulation. This substance named "Sympathin" is closely related to adrenalin. Sympathin is also discharged from the liver by stimulation of the sympathetic [107].

There are certain differences between adrenalin and sympathin which should be noted. Thus, sympathin does not dilate the iris, and its hypertensive effect is unaffected by ergotin. Cannon and Rosenblueth [108] explain these differences by assuming two kinds of sympathin, excitatory "Sympathin E" obtained from the liver, and inhibitory "Sympathin I" obtained from the cardio-pulmonary region. The sympatheticomimetic mediators are blocked by ergotoxine, atropine and yohimbine. The response to adrenalin is increased by various amino-acids, calcium salts and thyroxin. A change in the ratio of the blood electrolytes causes an alteration in the effects of adrenalin or sympathin. Both are synergized by calcium and antagonized by potassium.

Adrenalin acts upon the parasympathetic nervous system in a vicious circle, for the stimulation of the parasympathetic liberates acetylcholine, which stimulates the discharge of adrenalin, which in turn inhibits the parasympathetic. If the enzymatic inactivation of acetylcholine is inhibited by physostigmine, an increased discharge of adrenalin occurs.

### **Vascular System**

Adrenalin causes increase in blood-pressure by constriction of the peripheral blood-vessels, especially the arterioles and capillaries. It also constricts the lymphatics and lymph nodes [109]. Although most arteries respond to adrenalin by contraction, the cerebral, pulmonary and coronary arteries react by dilatation. The most marked constrictive effect is elicited in the renal artery and particularly in the efferent capillaries of the glomeruli. Very small amounts of adrenalin suffice to produce this result. A similar effect is produced in the hepatic intestinal arteries, but requires a higher concentration of adrenalin. The pulmonary arteries react differently to adrenalin. Following a brief constriction these vessels dilate; though if large doses are used the constriction is maintained [110]. The coronary artery does not respond to adrenalin even with a transient constriction.

Pheochromocytomas of the adrenals produce paroxysmal hypertension, possibly accompanied by hyperglycaemia and glycosuria. The salient symptoms associated with the attack are pallor of the face, tingling of the extremities, feeling of cold, headache, palpitation, tachycardia, anginal pain, nausea, restlessness, and increased pulse rate and blood pressure [247-50]. The adrenalin content of the blood during attacks was found in 2 cases to be increased from 4 to 5 times [251].

The administration of adrenalin occasionally results in lowering of the blood-pressure, which is due to adrenoxin, a vasodilator compound formed from adrenalin or catechol oxidase. Adrenoxin has this vasodilator effect, however, only when adrenalin is given in low concentrations.

### **Muscular System**

Adrenalin increases and accelerates the contractions of the heart muscle, lowers the muscle tone of the large intestine, stomach and oesophagus, inhibits peristalsis, and constricts the pyloric, ileo-caecal and internal anal sphincters.

The non-gravid uterine musculature in experimental animals and the human female is contracted by adrenalin, whereas the pregnant

human uterus is inhibited. According to Brown and Wilder [111] 1-2 minims doses of adrenalin solution 1 : 1,000 in saline do not produce uterine relaxation; or if they do, the relaxation is always preceded by a period of increased activity. This is the case in the human uterus spontaneously in labour, in the puerperal uterus exhibiting spontaneous rhythm, and in the puerperal uterus with an oxitocically induced rhythm.

Adrenalin given by the intravenous route causes dilatation of the pupil, retraction of the eyelids, protrusion of the eyeballs and contraction of the smooth muscles of the orbit. The diagnostic application of adrenalin to the conjunctiva, producing mydriasis in cases of diabetes and Graves's disease, has not proved reliable.

Administration of adrenalin produces marked relaxation of the bronchial musculature; on the striated musculature in a state of exhaustion, however, the effect is most stimulating.

### **The Effects of Adrenalin on Glands**

Adrenalin exerts a stimulating effect on many glands. Administered subcutaneously or intravenously it stimulates the secretion of the salivary glands, stomach and liver. The sweat glands, however, do not respond. Large doses inhibit pancreatic secretion. Eskin [112] showed, in the rabbit, that injections of adrenalin inhibit the release of luteinizing hormone from the anterior lobe of the pituitary gland. With simultaneous injections of adrenalin, both oestrogen and progestogen fail to elicit the release of gonadotrophic hormone from the anterior lobe of the pituitary gland.

### **Basal Metabolism**

Subcutaneous injections of adrenalin raises the basal metabolism in most animals, 1 mg. raising it in man by 50 per cent for a few hours. Adrenalin also accelerates respiration and, injected subcutaneously or intraperitoneally, raises body temperature by small amounts; larger doses may cause a drop in temperature. Finally, adrenalin has been found to interrupt hibernation.

### **Carbohydrate Metabolism**

Carbohydrate metabolism is markedly influenced by adrenalin, for subcutaneous injections produce glycosuria preceded by hyperglycaemia, which is caused by depletion of the liver glycogen and to a small degree by a decrease in muscle glycogen. The relationship of adrenalin to insulin is one of antagonism. Denervation of the adrenals increases hypoglycaemia, while hypoglycaemia causes discharge of



adrenalin from the adrenals. The thyroid hormone sensitizes the organism to adrenalin, and pituitrin inhibits its glycosuric effect. The rôle of the anterior lobe of the pituitary gland in the response to adrenalin is one of synergism; it acts by making the liver ready to mobilize its glycogen upon adrenal stimulation. Injection of adrenalin in the hypophysectomized animal or stimulation of the adrenals increases the discharge of adrenalin, but does not cause hyperglycaemia [113].

### **Toxic Effects**

Adrenalin in excessive amounts acts as a powerful poison. A toxic dose administered by the intravenous route may cause almost immediate death by spastic paralysis of the heart. Repeated injections produce cardiac hypertrophy. Lesions of the aorta develop after repeated intravenous injections.

### **Control of Adrenalin Secretion**

The secretion of adrenalin is regulated by the autonomic nervous system. Stimulation of the splanchnic nervous system increases the secretion of adrenalin. Section of the splanchnic nerves decreases the output of adrenalin. The physiological stimuli which reach the adrenal through the splanchnic nerves arise from higher centres, located below the fourth ventricle.

### **Factors Stimulating Adrenalin Secretion**

Emotional impulses act as powerful secretory stimuli. Exposure to cold, fever, muscular activity, anoxia, and drugs such as morphine, caffeine, theobromine, picrotoxin and nicotine, are strong stimuli to the medulla. Nicotine inhaled in the course of smoking causes mild hyperglycaemia, apparently as the result of adrenalin discharge [114]. Physostigmine and acetylcholine, or its mother-substance choline, stimulate the medulla by acting upon the autonomic nervous system. Atropine and ergotamine inhibit the secretion of adrenalin.

In emergency, sudden demands on muscular activity, danger, emotional stress, sudden changes in atmosphere or blood volume, the adrenal medulla reacts with intense secretion of adrenalin. Burns or scalds of the skin act as powerful stimulants to the medulla [115].

### **Protective Effects**

Adrenalin plays an important part in augmenting the resistance to infections and in their treatment. Its administration in malaria causes

rapid disappearance of the parasites from the blood, preceded by retraction of the spleen [116].

Based on the values obtained in the dog, the human subject weighing 60 kilograms would secrete 0.0042 mg. adrenalin per minute, or a total of 6 grams during the course of the day [117].

#### SUMMARY OF DIFFERENT EFFECTS PRODUCED BY THE ADRENAL CORTX AND THE MEDULLA

<i>Function</i>	<i>Cortex</i>	<i>Medulla</i>
Maintenance of Life . . . . .	Necessary	Not necessary
Oxygen consumption . . . . .	Increase	Increase
Permeability of Membranes . . . . .	Decrease	Decrease
Potassium (Plasma) . . . . .	Decrease	Increase
Potassium (Tissue) . . . . .	Increase	Decrease
Blood Sugar . . . . .	Increase	Increase
Glycogen (Tissue) . . . . .	Increase	Decrease
Fat (Tissue) . . . . .	Increase	Increase
Blood Pressure . . . . .	Increase	Increase
Renal Function . . . . .	Increase	Increase
Activity of Striated Muscle . . . . .	Increase	Increase
Effect on Thyroid . . . . .	Inhibitory	Synergistic
Effect on Pancreas . . . . .	Inhibitory	Inhibitory
Effect on Anterior Pituitary . . . . .	Synergistic	Synergistic
Effect on Posterior Pituitary . . . . .	Antagonistic	Antagonistic
Effect on Gonads . . . . .	Androgenic	Inhibitory
Effect on Thymus . . . . .	Inhibitory	Inhibitory

Goldzieher [118].

#### THE RELATIONSHIP OF THE SUPRARENALS TO OTHER ENDOCRINE GLANDS (*see Figs. 1 and 4*)

##### Anterior Pituitary Lobe

The adrenal gland receives its main stimulus from the anterior lobe of the pituitary. Experimental destruction of the anterior lobe causes considerable atrophy of the adrenal cortex [119]. In the hypophysectomized rat, the adrenals decrease within 6 days to half their size and atrophy completely within 1 month. An interesting finding in this connection is the hypoplasia of the cortex in cases of anencephaly.

The corticotrophic hormone exerts its effect not only on the atrophic adrenals of the hypophysectomized animal, but also upon the normal gland. According to Davidson [120], the corticotrophic hormone increases the size and weight of the normal gland by about 300 per cent as compared with 700 per cent in the hypophysectomized animal.

The eosinophilic cells of the anterior pituitary lobe are apparently the site of corticotrophic hormone production, for hypertrophy of the adrenals is almost constant in cases of acromegaly. Against this view may be cited the fact that cortical hyperplasia or adenomata of the cortex are frequently associated with a basophil adenoma of the anterior pituitary. Recently, however, a number of cases of Cushing's Syndrome without pituitary basophilism [121, 122, 123, 124], and cases of basophilic tumours without Cushing's Syndrome [125], have been reported. The changes in the basophil cells associated with Cushing's Syndrome consist of cytoplasmic hyalinization, disappearance of the basophilic granules, ballooning of the nuclei, excessive vacuolization, tendency to multinucleation and general enlargement of the cells [126]. These changes may occur without a basophil cell adenoma, or if the latter is present hyalinization occurs only in the extra-adenomatous basophilic cells.

Crooke [126] concluded from his observations that the hyaline changes in the basophil cells are an expression of altered physiological activity, and the fundamental factor in the causation of adrenal cortical hyperfunction. This interpretation has been questioned by Beveringhaus and others [127, 128], who believe that the hyaline changes signify degeneration following a period of hyperactivity. This preceding period of increased basophil activity is probably due to hyperfunction of the adrenal cortex, which suppresses the activity of the eosinophils, producing the changes described in the basophils. Adrenalectomy or pathological destruction of the adrenals causes a decrease in the number of the basophil cells [129, 130], whereas implantation of the adrenal cortex causes an increase in the total number of basophil cells, due to the appearance of degenerated cells and a decrease in the eosinophils [129] (see Figs. 12 and 13).

Adrenalectomy carried out simultaneously with castration inhibits or slows down the development of castration cells, and produces a decrease in the amount of eosinophilic cells [130].

Administration of thiouracil in the rat produces a decrease in the number of eosinophils and enlargement with vacuolization of the basophil cells, accompanied by involution of the adrenal cortex [131]. Injections of cortical extract produce atrophy of the adrenal cortex, probably by suppression of the pituitary corticotrophic function, in the same way as unilateral tumours of the cortex cause atrophy of the contralateral gland [132].

There is evidence that the cortical function depends only partly on the corticotrophic hormone of the pituitary and remains partly independent (see Chapter I). In the hypophysectomized



animal, the adrenal atrophies, but the animal does not succumb. Adrenalectomy, on the other hand, is incompatible with life. The blood of the hypophysectomized animal shows no significant changes of sodium chloride concentration, in spite of the advanced atrophy of the adrenals [133]. This suggests that the pituitary corticotrophic hormone only slightly controls salt and water metabolism.

There is no convincing evidence that the function of the medulla is influenced by the pituitary. However, the effects of adrenalin are modified by the pituitary gland, for the glycogenolytic effect of adrenalin cannot occur in the absence of the anterior lobe [134]. Injections of desoxycorticosterone acetate cause, in addition to atrophy of the cortex, degenerative changes of the medulla [135].

### **Posterior Pituitary Lobe**

The relation of the adrenal cortex to the posterior lobe of the pituitary is one of synergism in the maintenance of normal water balance. If, however, the balance between the posterior lobe of the pituitary and the adrenal cortex is disturbed by failure of the one or the other gland, an antagonism is created which produces hyperfunction of the contralateral gland in the sense that the posterior lobe exerts an anti-diuretic and the adrenal a diuretic effect.

Polyuria produced by hypophysectomy is checked by adrenalectomy [136]. Adrenalectomy, on the other hand, increases the anti-diuretic substance of pituitary origin in the urine shortly after the removal of the adrenals. Injection of desoxycorticosterone acetate increases water intake and output, and reduces sodium and chloride excretion.

An antagonism between adrenalin and pituitrin exists in the action on the uterine muscle, for the inhibiting effect of adrenalin upon the myometrium is reversed by posterior lobe extracts.

Adrenalin is synergic to pitressin, in so far as pitressin sensitizes the tissues to the effect of adrenalin. Both substances given separately have a hyperglycaemic effect; their combined administration, however, inhibits the increase in sugar.

### **Thyroid Gland**

Thyroid feeding produces enlargement of the adrenals. Compensatory unilateral hypertrophy of the adrenals after adrenalectomy, and hypertrophy due to physical exercise or administration of oestrogens, is augmented by thyroid feeding. Swann [137] has shown that this effect is brought about through the pituitary, since it is absent in the hypophysectomized animal.

Rochlin [129] demonstrated that the action of the adrenal cortex on the thyroid is not mediated through the pituitary, for adrenalectomy causes stimulation of the thyroid gland with no increase of thyrotrophic function. After implantation of the adrenal cortex, a marked inhibition of the thyroid gland is observed with, at the same time, increased thyrotrophic function of the pituitary. Prolonged action of the thyroid hormone causes adrenal cortical atrophy. In hyperthyroidism, or Graves's disease, the adrenals are smaller and lighter, due to the atrophy and lipoid depletion of the cortex [139, 140, 141], whereas the medulla appears to be normal [142].

The frequently noted coincidence of hyperthyroidism and Addison's disease may be explained by the fact that hyperthyroidism, representing the primary factor, causes Addison's disease, by prolonged stimulation of the adrenal cortex and its subsequent failure. On the other hand, patients suffering from Addison's disease may suddenly develop symptoms of hyperthyroidism, the thyroid being relieved from the damping effect of the adrenal cortex [143].

The inhibiting action of the adrenal cortical hormone on the thyroid is demonstrated in the experimental animal by adrenalectomy, which produces a rise in the basal metabolism [144, 145]. On the other hand, states of hyperthyroidism or Graves's disease are favourably influenced by administration of the cortical hormone [146, 147, 152-4]. According to Goldzieher [142] cortical therapy is a most valuable aid in the medical treatment of hyperthyroidism (see Chapter III).

The relationship between the adrenal medulla and the thyroid is one of synergism. The thyroidectomized animal does not respond adequately to adrenalin, whereas prolonged administration of the thyroid hormone increases the pressor and glycaemic response to adrenalin; injection of adrenalin in the thyroid-fed animal increases oxygen consumption, whereas the opposite takes place in the thyroidectomized animal [148]. Thyroid hormone and adrenalin liberate potassium from the tissues and the effects of both are increased if the potassium-calcium ratio increases.

### Parathyroid

In parathyroid insufficiency the response to adrenalin is diminished, whereas convulsions of tetany increase the discharge of adrenalin from the medulla.

An antagonism between the parathyroid and the adrenal cortex is suggested by the similar symptomatology found in hyperparathyroidism and acute adrenal failure. Both are characterized by a loss of

sodium chloride and dehydration, followed by oliguria and azotaemia [149].

### Thymus

The relationship of the adrenal gland to the thymus is one of antagonism. Persistence or hyperplasia of the thymus is met with in adrenal cortical deficiency. It seems that the medulla takes part in the antagonism to the thymus, for congenital hypoplasia of the medulla is consistently associated with hyperplasia of the thymus and the whole lymphatic system.

According to Selye, the "alarm reaction" which is associated with a sudden increase in the secretion of the cortical hormone is followed by involution of the thymus. Related steroids, such as testosterone, oestrogen and progesterone are also capable of producing involution of the thymus [150] (see Chapter X).

### Pancreas

There is an antagonism between the cortical hormone and insulin. Adrenalin, too, is antagonistic to insulin.

Removal of the adrenals in the experimental animal increases responsiveness to insulin, for sub-threshold doses of insulin produce severe hypoglycaemia and convulsions. Conversely, larger doses of adrenalin neutralize the hypoglycaemic effects of insulin. Hyperglycaemia and glycosuria, produced by increased secretion of the medulla or injection of adrenalin, are lowered by insulin, which increases the oxidation of sugar and inhibits glycogenolysis. The antagonism between adrenalin and insulin concerns carbohydrate metabolism only, for insulin does not inhibit the vasoconstrictor effects of adrenalin.

Although both the cortical hormone and adrenalin are antagonistic to insulin, there exists a difference between them, for the main function of the cortical hormone is maintenance of the hepatic store of glycogen and production of new glycogen from protein, whereas adrenalin increases the blood sugar by glycogenolysis and supplies lactic acid from the musculature for reconversion to glycogen in the liver.

Diabetes produced by alloxan in rats shows marked reduction or complete disappearance after adrenalectomy [151]. Dosne [41] demonstrated, in experimental diabetes of the toad following subtotal pancreatectomy, that simultaneous removal of the anterior lobe of the hypophysis prevented the appearance of pancreatic diabetes; but that subsequent injections of anterior pituitary tissue provoked hyperglycaemia. Adrenalectomy attenuated the state of pancreatic diabetes



which could be made to reappear by injection of anterior pituitary lobe extracts. Extirpation of the thyroid in no way modified the production of pancreatic diabetes.

In rabbits [43], mice [44] and pigeons [45, 46], adrenal hypertrophy has been reported after prolonged administration of insulin (see Chapter VI).

### Gonads

The adrenal cortex produces oestrogens and androgens. Androgens not only fail to stimulate the adrenal cortex but may produce hypoplasia of the cortex. Oestrogens, both natural and synthetic, stimulate the cortical tissue. Excessive doses may cause degenerative changes, however [45].

Changes in the adrenal cortex during the normal menstrual cycle are reflected by gain of weight and reduction in the excretion of sodium chloride and water during the intermenstrual and premenstrual periods. The onset of menstruation is associated with increased renal excretion of sodium chloride and water. Enlargement of the cortex occurs also during pregnancy.

It has been suggested that the effects of oestrogen on the adrenal cortex are mediated through the pituitary by increase of the corticotropic hormone, since oestrogen does not produce hypertrophy of the adrenals in hypophysectomized rats [167]. If androgens are administered simultaneously with oestrogen, the hypertrophy of the pituitary and cortex is inhibited [83]. Oestrogen supplied simultaneously with thyroid has a cumulative effect on the adrenal cortex, eliciting increased function [89]. Progestogen, unlike oestrogen, produces hypertrophy with decreased function of the cortex.

Increased production of androgens by the adrenal cortex causes extinction of ovarian function, as shown by quantitative studies of urinary 17-ketosteroids [160] (see Chapter IX).

Allen and Bourne [161] recently obtained an extract of the whole adrenal gland, which is chemically distinct from the life sustaining principle, and which induces luteinization and a considerable degree of endometrial hypertrophy. They named it adrenolutein. Large doses of desoxycorticosterone, one of the active compounds of the adrenal cortex, induces progestational changes in the endometrium of the rabbit and the monkey [162, 163]. Pregnanediol, the chief product of progestogen metabolism, appears in unusually large amounts in the urine of patients with adrenal virilism [14, 165], and some patients with adrenal cortical neoplasm excrete large quantities of oestrogen.

Adrenalectomy causes a drop in the gonadotrophic hormone content of the anterior pituitary gland, and a decrease in the size and number of eosinophils and basophils [166]. On the other hand, large doses of cortical extract cause hypertrophy of the anterior pituitary gland (see Figs. 12 and 13).

In the male, castration produces hypertrophy of the adrenal cortex, which may be prevented by the administration of androgens [89] (see Chapter IX).

### Lactation

The adrenal cortex is essential for the maintenance of normal lactation. Opinion is divided on whether the adrenal cortex secretes a specific lactogenic hormone, or whether it activates the lactogenic hormone elaborated by the anterior lobe of the pituitary.

There is evidence that very potent preparations of lactogenic hormone are powerless to induce the secretion of milk in hypophysectomized guinea pigs, except by the aid of adrenotrophic hormone or adrenal cortical extract [98].

### THE ADRENALS AND VITAMINS

The experimental animal develops considerable cortical hyperplasia in the course of increased muscular activity. This enlargement can be prevented if five times the normally required amount of vitamins is administered [169]. The adrenals take part in the storage of vitamin A and their function may possibly be affected by vitamin A deficiency [252, 253].

Marked enlargement of the adrenals has been observed in cases of beri-beri. According to Verzar and Peter [168] this may reach at least 50 per cent in pigeons, rats and rabbits. Some of it is due to oedema and some to the increased lipoid load.

Vitamin B deficiency disorders do not always improve after vitamin B administration, the vitamin not being retained unless a cortical extract is also administered [170]. There is a like relationship between the adrenal cortex and other vitamins of the B complex, i.e. riboflavin, pantothenic acid, pyridoxin, nicotinic acid and *p*-amino benzoic acid [171].

Riboflavin deficiency causes decrease in adrenal weight, and Goldzieher suggests that the symptoms of riboflavin deficiency observed in Addison's disease may be due to the fact that riboflavin has been used up in order to compensate for the cortical insufficiency. Adrenal haemorrhage has been noted in animals kept on a riboflavin deficient diet. Pantothenic acid deficiency causes haemorrhagic necrosis in 60



per cent of deficient rats [172]. According to Unna and Antopol [173], the administration of pyridoxin in excessive doses produces adrenal changes similar to those observed in pantothenic acid deficiency.

A condition similar to pellagra develops in adrenalectomized rats and improves under treatment with nicotinamide. McCarrison and Hertsenberg [174, 175] observed adrenal changes akin to those in human pellagra if the diet was lacking in nicotinic acid. The symptoms which develop in pellagra resemble those of Addison's disease, particularly pigmentation of the skin, hypotension and asthenia [176].

Vitamin C serves to stabilize both the medullary and the cortical hormone. Its function in the regulation of skin pigmentation has been demonstrated by Szent Györgyi and others [254-7, 45, 49, 164] who have shown that Addison's disease is associated with vitamin C deficiency. The skin pigmentation diminishes strikingly after oral or parenteral administration of vitamin C. Rothman [256] has shown that melanin is reduced by vitamin C to water-soluble lighter pigments. He has noted a bleaching of the pigmented areas after giving 500 mg. of vitamin C daily by mouth for 6 months.

#### STANDARDIZATION

##### **Cortical Extracts and Desoxycorticosterone Acetate**

The activity of potent cortical extracts derived from animal adrenal glands is due to a mixture of several of the adrenal steroid compounds [95].

The commercially available cortical extracts are not standardized against known quantities of crystalline hormones, and it is therefore obvious that preparations may vary widely in their potency and in their content of active adrenal principles.

The cortical extract is standardized either in terms of the quantity of fresh gland from which it is derived (e.g., 1 c.c. of extract from 40, 50 or 75 grams of fresh gland) or by assay of its physiological activity.

Several methods of assay are used:

1. Survival of adrenalectomized rats in a low environmental temperature [178, 212].

This method is not specific for the identification of any particular compound.

2. Survival of the adrenalectomized animal in a low atmospheric pressure [179].

This is suitable for cortical extract and desoxycorticosterone acetate.



3. Growth of young rats [180].

This method is unsuitable, since Compound E or corticosterone retard growth.

4. Maintenance of a normal condition in adrenalectomized dogs [181].

For this method crude extracts as well as various crystalline compounds and the amorphous fraction are suitable. However, the amounts of the various substances needed vary considerably.

5. Influence on the toxic action of potassium salts [182] and typhoid vaccine [213].

Suitable for desoxycorticosterone acetate and compounds possessing oxygen atom at C-11.

6. Deposition of glycogen in the liver of fasting adrenalectomized rats [45, 183, 211, 257].

17-Hydroxy-11-dehydrocorticosterone and related compounds cause deposition of glycogen.

7. Anti-insulin effect [184].

Administration of cortical extracts to mice renders them resistant to small amounts of insulin injected intraperitoneally.

8. Stimulation of muscle.

(a) The extract to be assayed is administered for some days to the adrenalectomized rat before the response of the muscle to stimulation is determined. Crude extract of desoxycorticosterone acetate restores the normal response. Other substances produce a feeble response [185].

(b) Corticosterone and related compounds prevent exhaustion of the anaesthetized adrenalectomized animal under continuous stimulation of its muscles [186].

9. Fall in level of blood-cholesterol and other lipoids [187].

Intravenous injection of interrenin in the rabbit produces a sharp fall in the level of blood cholesterol and other lipoids.

### Adrenalin

A number of methods are described for the assay of epinephrine or adrenalin.

1. The intestinal or uterine strip method.
2. The blood-pressure method.

3. The perfusion method.
4. The excised eye method.
5. The action on the denervated heart.

#### PREPARATIONS FOR CLINICAL USE AND ADMINISTRATION

More than twenty crystalline derivatives have been isolated from the adrenal cortex, but only seven are capable of replacing cortical function. The most active crystalline derivative is a steroid compound with the formula  $C_{19-21}H_{28-30}O_5$ .

Recently MacBryde and de la Blazé [188] investigated the effects of porcine adrenal cortex extracts on carbohydrate metabolism and work-capacity in Addison's disease. The extracts used contained 40 rat units per c.c. It was found that they had a direct action on the carbohydrate metabolism, and that this was greater than that of bovine adrenal cortex extracts. One c.c. of the porcine extract represents approximately the same survival growth potency as 10 c.c. of bovine extract. When given intramuscularly in these proportions, usually in doses of 2 c.c. and 20 c.c. respectively, the porcine extract tends to correct the blood-sugar abnormalities of Addison's disease, whereas the bovine extract has little comparable effect. The porcine extract seems not only to mobilize glycogen from the liver but also to increase the utilization of glucose by the muscles.

The majority of preparations now available commercially are extracts of fresh bovine adrenals. Ingle [189] demonstrated that although small amounts of bovine adrenal cortex will maintain life in adrenalectomized animals when conditions for survival are optimal, relatively massive doses are required to maintain normal resistance to stress.

#### Cortical Extracts

for intravenous or subcutaneous injection:

- 1 c.c. is equivalent to 25 or 50 dog units
- 1 c.c. is equivalent to 40 grams of fresh gland
- 1 c.c. is equivalent to 50 grams of fresh gland
- 1 c.c. is equivalent to 75 grams of fresh gland

#### Desoxycorticosterone Acetate

Desoxycorticosterone acetate is synthesized from stigmasterol. It is available in propylene glycol in oil for intramuscular injection, and in the form of tablets for sublingual use, and of pellets for implantation.

*Desoxycorticosterone Acetate* in oil solution for intramuscular use: 1 c.c. contains 5–10 mg. of the compound.

*Desoxycorticosterone Acetate* pellets for implantation (chemically pure crystalline); usual size 125 mg.

Thorn [94] recommends the posterior infrascapular region for pellet implantation. A transverse incision of 2–4 cm. is made a few centimetres below the inferior spine of the scapula, and by blunt dissection a number of small pockets, 2–3 cm. in depth, are prepared in the subcutaneous tissue. Di Maio and Bird [191] modified this technique by using a trocar to implant the pellets in a row at some distance from the margin of the incision. This technique has the advantage of needing only a small incision. One pellet (125 mg.) implanted subcutaneously will substitute successfully for each 0.5 mg. of hormone required daily for a period of 9–15 months [94].

The absorption rate of desoxycorticosterone acetate, administered as 75 mg. cylindroid pellets implanted subcutaneously in patients with Addison's disease, varies from 0.14 to 0.35 mg. per pellet daily. The size and shape of the pellet considerably modify the absorption rate [192]. Shipley [193] found that the average number of 75-mg. pellets initially required in Addisonian patients is 4–6, and that the effective life of each pellet is from 9 to 10 months.

According to Koepf and Kibler [49] the average daily hormone absorption from a 125-mg. pellet by human subjects is 0.35 mg. The average duration of each implant was 334 days. Approximately 60 per cent of the daily dose of desoxycorticosterone acetate in oil is required if the compound is given in pellet form. To determine the number of pellets to implant the following formula is recommended:

$$\text{Pellet needed} = \frac{\text{daily dose desoxycorticosterone acetate in oil} \times 0.60}{0.35}$$

### Epinephrine

This is available under the Trade Mark "Adrenalin" in 1 : 1000 solution. The usual dosage is 0.5–1 c.c. subcutaneously: as an anaesthetic it is given by intracardial injection.

A preparation of adrenalin in vegetable oil 1 : 500 has been prepared for slow action. Its advantage is that injections do not have to be made so frequently. The usual dosage recommended is 0.22–1.5 c.c. [194].

For nasal spray, a solution of 1 : 100 is available.



### *Contraindications*

Arteriosclerosis.

Hypertension.

Hyperthyroidism.

Cardiac dilatation and coronary disease.

(Pregnancy does not in itself contraindicate the use of the drug.)

## CLINICAL APPLICATION AND APPROXIMATE DOSAGES

### **Cortical Extract and Desoxycorticosterone Acetate**

#### **Addison's Disease**

Desoxycorticosterone acetate in propylene glycol 10 mg. daily is ineffective [195], and the method is cumbersome and wasteful [196].

Desoxycorticosterone acetate sublabially or sublingually is effective but wasteful [196, 197], and the dose has to be one-third higher than for intramuscular administration [198].

Intramuscular administration, though very effective, is not economical; and it is also far less convenient than implantation, which proves to be the method of choice on the grounds of both economy and convenience [196, 199].

#### **Severe Addisonian Crisis**

##### **Initial treatment:**

Cortical extract: 20–30 c.c. to be given initially with immediate intravenous administration of saline and glucose; followed by 10 c.c. intramuscularly every 6 hours, the dose being gradually reduced as the crisis subsides.

Dunlop [197]

##### **Maintenance:**

Cortical extract: In severe cases, 10 c.c. of cortical extract given intramuscularly daily.

Thorn [94]

Desoxycorticosterone Acetate: 5 mg. intramuscularly daily.

Mild cases may do well on as little as 5 mg. desoxycorticosterone acetate per week. A preferable method recommended for maintenance is implantation of 200–400 mg. pellets into the deep subcutaneous tissue of the abdomen, by which the effect of desoxycorticosterone acetate is maintained from 6 to 12 months [197]. Implantation should not be attempted until the patient's daily need has been well standardized by intramuscular injections.

### **Waterhouse-Friederichsen Syndrome**

Treatment as for severe Addisonian crisis.

### **Acute Adrenal Insufficiency in Children**

Cortical extract should be administered early and in liberal amounts as soon as its use becomes urgent—whenever a child affected by an acute infectious disease appears to become toxic.

In cases of diphtheria, one should not wait until this stage is reached, but cortical therapy should be started immediately the diagnosis is established [200].

### **Chronic Adrenal Insufficiency**

Cortical extract: 5 c.c. intravenously followed by 2.5 c.c. intramuscularly at intervals and in amounts adjusted to the severity of the symptoms.

Desoxycorticosterone acetate: This decreases the amount of cortical extract needed and should therefore be used in conjunction with the extract. Not more than 1 mg. of desoxycorticosterone acetate should be used at a time and too frequent injections should be avoided [201].

Desoxycorticosterone Acetate Pellets: 4–6 pellets, each of 75 mg., implanted subcutaneously. The effect lasts for 6–12 months [193, 197].

Adrenal cortical tissue obtained from a case of adrenal virilism has been successfully transplanted into a patient suffering from chronic adrenal insufficiency [202]. Similar successful transplantations have been reported by Katz [203] and Auslander [204] and recently by Broster and Gardiner-Hill [59].

### **Beneficial Effects of Cortical Extract and Desoxycorticosterone Acetate in Other Conditions Hyperemesis Gravidarum**

Cortical extract 1 c.c. 3 times daily [155–9].

Several investigators have noted temporary inhibition of adrenal cortical function following therapeutic doses of adrenal cortical extract, and a “compensatory atrophy” after large doses [158].

### **Peripheral Vascular Diseases**

Desoxycorticosterone acetate: According to Sirota [206] the beneficial physiological effects of hypertonic solutions of sodium chloride

intravenously in thrombo-angiitis obliterans and in arteriosclerosis obliterans can, within limits, be obtained by intramuscular injections of desoxycorticosterone acetate 10 mg. once or twice weekly.

### **Roentgen Sickness**

Desoxycorticosterone acetate:

Administration of desoxycorticosterone acetate resulted in immediate improvement in nearly every case [207].

### **Gastric Ulcer**

Desoxycorticosterone acetate: 20–35 mg. daily intramuscular injections.

This dosage relieves pain in 2 or 3 days. Case reports and radiographs have confirmed rapid healing. It is suggested that besides improving the asthenic hypotonic vaso-labile constitution of the patients, desoxycorticosterone acetate has a direct action on the gastrointestinal mucous membrane [208].

### **Duodenal Ulcer**

Treatment as for gastric ulcer.

### **Whooping Cough (pertrussis)**

Cortical extract:  $\frac{1}{2}$ –1 c.c. 4 to 6 times was administered intramuscularly for 2 or 3 days to 15 patients during a very severe epidemic. The severity and duration of attacks was markedly diminished; and the sooner the cortical extract was given after the onset of symptoms, the shorter was the course of the disease [209].

### **Burns**

Cortical extract: single doses of 10–30 c.c. (250–750 dog units) according to haemoconcentration.

There have been reports of the successful use of cortical extracts in burns associated with haemoconcentration, electrolytic changes, acidosis, hypoproteinaemia, sodium loss and hyperpotassaemia [210].

### **Schizophrenia**

Cortical extract: 1 c.c. 3 times a week.

Manic depressive patients were treated with adrenal cortical extract 1 c.c. 3 times a week with beneficial effects [190].



### Miscellaneous Uses

Whole adrenal gland 8-15 mg. 1-3 times daily has been used with beneficial results in the following conditions:

glaucoma, progressive myopia, hay fever, asthma, vernal catarrh and eczema.

Haseltine [177]

### Adrenalin 1 : 1000

Adrenalin is used for producing a bloodless operative site in conjunction with local or spinal anaesthesia. It also serves to maintain the high local concentration of anaesthetic.

Intravenous or intramuscular injections of adrenalin are rarely indicated, and by the oral route it is ineffective. There is no advantage in combining it with pituitrin.

### Resuscitation

Adrenalin hydrochloride is injected intracardially by inserting a 6-10-cm. needle in the fourth intercostal space at the upper border of the fifth rib close to the sternum. The needle is pushed in to a depth of approximately 4-5 cm. The dose is 1 c.c.

### Bronchial Asthma or "Status Asthmaticus"

Adrenalin hydrochloride: 2 minims every 5 minutes for half an hour (Beaumont)

or

0.1-0.15 c.c. repeated at intervals every 30 minutes for 2 or 3 doses (Stirling).

### Haemorrhage

Adrenalin hydrochloride: Local application.

### Congestion of Mucous Membranes

Adrenalin hydrochloride: Local application.

### Urticaria

Adrenalin hydrochloride: 2 minims every 5 minutes for half an hour (Beaumont)

or

0.1-0.15 c.c. repeated at intervals every 30 minutes for 2 or 3 doses (Stirling).

### Angioneurotic Oedema

Adrenalin hydrochloride: 2 minims every 5 minutes for half an hour.

### Serum Sickness

Adrenalin hydrochloride: 2 minims every 5 minutes for half an hour (Beaumont)

or

0.1–0.15 c.c. repeated at intervals every 30 minutes for 2 or 3 doses (Stirling).

### Nitritoid Crisis following Injections of Arsephenamine

Adrenalin 1 : 1000: 2 c.c. subcutaneously every 2 hours until improvement is noted.

### Acute Circulatory Failure

Adrenalin 1 : 1000: 0.5 c.c., repeated after a few minutes if relief has not been obtained.

### Hypoglycaemia

Adrenalin 1 : 1000: 0.5–1 c.c.

### Heart Block (Adams-Stokes Syndrome)

Adrenalin hydrochloride 0.3–0.6 c.c. subcutaneously.

## BIBLIOGRAPHY

1. SWINYARD, C. A. *Anat. Rec.*, **68**, 417, 1937.
2. MACFARLAND, M. E. *J. Exper. Biol.*, **95**, 345, 1944.
3. BENNETT, H. S. *Am. J. Anat.*, **69**, 333, 1941.
4. DOSNE, C., and DALTON, A. J. *Anat. Rec.*, **80**, 211, 1941.
5. SARASON, E. C. *Arch. Int. Med.*, **71**, 702, 1943.
6. BOURNE, G. *Austral. J. Ex. Biol.*, **12**, 123, 1934.
7. SZENT-GYÖRGYI, A. *Biochem. J.*, **22**, 1387, 1928.
8. BENNETT, H. S. *Am. J. Anat.*, **67**, 151, 1940.
9. MILLER, E. *Am. J. Anat.*, **40**, 251, 1922.
10. LEBLOND, C. P., and GARDINER, W. E. *Anat. Rec.*, **72**, 119, 1938.
11. BROSTER, L. R., and VINES, H. W. C. "Adrenal Cortex", H. K. Lewis, London, 1933.
12. BROSTER, L. R., CLIFFORD, ALLEN, VINES, H. W. C., PATTERSON, JOCELYN, GREENWOOD, ALAN W., MARRIAN, G. F., BUTLER, G. C. "Adrenal Cortex and Intersexuality", Chapman & Hall, London, 1938.

13. REICHSTEIN, T. *Helv. Chim. Acta.*, **19**, 203, 1936.
14. BUTLER, G. C., and MARRIAN, G. F. *J. Biol. Chem.*, **119**, 565, 1938.
15. REICHSTEIN, T., and EUQ, J. *Helv. Chim. Acta.*, **21**, 1197, 1938.
16. PFIFFNER, J. J., and NORTH, H. B. *J. Biol. Chem.*, **139**, 855, 1941.
17. BEALL, D., and REICHSTEIN, T. *Nature*, **142**, 479, 1938.
18. BEALL, D. *Nature*, **144**, 76, 1939; *J. Endocrinol.*, **2**, 81, 1940.
19. GOLDZIEHER, M. M. "The Adrenals in Health and Disease", F. A. Davis Co., Philadelphia, 1944.
20. SANDSTROM, E. S., and MICHAELS, G. "Adrenal Cortex in Adaptation to Altitude, Climate and Cancer", University of California Press, Berkeley and Los Angeles, 1942.
21. EASTMAN, N. J. *Bull. Johns Hopkins Hospital*, **47**, 221, 1930.
22. ESTRADA, O. P. *Ref. Soc. Argent. Biol.*, **2**, 367, 1926.
23. HIMWICH, H. E., FAZEKAS, J. G., BARKER, S. D., and HURLBURT, F. H. *Am. J. Physiol.*, **110**, 348, 1934.
24. BRYAN, A. H., and RICKETTS, H. T. *J. Clin. Endocrinol.*, **4**, 450, 1944.
25. MILLER, R. A., and RIDDLE, O. *Proc. Soc. Exper. Biol. & Med.*, **41**, 518, 1939; *ibid.*, **47**, 449, 1941.
26. GERSH, I., and GROLLMAN, A. *Anat. Rec.*, **75**, 131, 1939.
27. SELYE, H., COLLIP, J. B., and THOMPSON, D. L. *Proc. Soc. Exper. Biol. & Med.*, **32**, 1377, 1935.
28. PARHON, C., and ZURGRAVU, G. *Arch. Internat. de Neurol.*, **35**, 273, 1913.
29. GOLDZIEHER, M. "Adrenal Glands in Health and Disease", p. 177, F. A. Davis Co., Philadelphia, 1944.
30. *Ibid.*, p. 170.
31. ANDERSON, J. A., and MURLIN, W. R. *J. Pediat.*, **21**, 326, 1942.
32. WELLS, B. B., and KENDALL, E. C. *Mayo Clin.*, **15**, 133, 1940.
33. SELYE, H., and BASSETT, L. *Proc. Soc. Exper. Biol. & Med.*, **45**, 272, 1940.
34. COREY, E. L., and BRITTON, S. W. *Am. J. Physiol.*, **133**, 511, 1941.
35. LOEB, R. T. *Science*, **76**, 68, 1932.
36. MENKIN, V. *Am. J. Physiol.*, **129**, 691, 1940.
37. GRAHAM, J. S. *Proc. Soc. Exper. Biol. & Med.*, **54**, 101, 1943.
38. HYMAN, C., and CHAMBERS, R. *Endocrinol.*, **32**, 310, 1943.
39. THORN, G. W., and EMERSON, K. *Am. Int. Med.*, **14**, 757, 1940.
40. GANES, R. G., and FRIEDGOOD, C. E. *Endocrinol.*, **36**, 62, 1945.
41. DOSNE, C. *Endocrinol.*, **33**, 224, 1943.
42. SPRAGUE, R. C., PRIESTLY, J. T., and DOCKERTY, M. B. *J. Clin. Endocrinol.*, **3**, 28, 1943.
43. SCHENCK, F., and LANGCKER, H. *Endocrinol.*, **16**, 305, 1935.
44. MICKLEITIS, B. *Anat. Rec.*, **89**, 337, 1940.
45. EGGLESTON, N. M., JOHNSTON, B. J., and DOBRINER, K. *Endocrinol.*, **38**, 197, 1946.
46. RIDDLE, O., HONEYWELL, H. E., and FISHER, W. S. *Am. J. Physiol.*, **68**, 461, 1924.



47. TEPPERMAN, J., ENGEL, F. L., and LONG, C. M. K. *Endocrinol.*, **32**, 373, 1943.
48. BOURNE, G., and ZUCKERMAN, S. *J. Clin. Endocrinol.*, **2**, 283, 1940.
49. KOEPF, G. F., and KIBLER, R. Twenty-Ninth Ann. Meet. of Assn. for Study of Internal Secretions, 1947.
50. VERZAR, F., and LASZT, L. *Biochem. Ztschr.*, **288**, 356, 1936.
51. INGLE, E. J. *J. Clin. Endocrinol.*, **3**, 603, 1943.
52. SWINGLE, W. W., and PFIFFNER, J. *Anat. Rec.*, **44**, 225, 1938; *Medicine*, **11**, 37, 1932.
53. MARINE, D., and BAUMANN, E. J. *Am. J. Physiol.*, **81**, 86, 1927.
54. OEHME, C. *Klin. Wchnschr.*, **15**, 512, 1936.
55. WHITE, A., and DOUGHERTY, T. F. *Endocrinol.*, **36**, 16, 1945.
56. WELLS, B. D., and KENDALL, E. C. *Mayo Clin.*, **16**, 113, 1941.
57. SLOT, W. J. B. *Act. Med. Scand.*, **89**, 371, 1936.
58. CROOKE, A. C., and CALLOW, R. K. *Quart. Med.*, **8**, 233, 1939.
59. BROSTER, L. R., and GARDINER-HILL, H. *Brit. Med. J.*, 4476, 570, 1946.
60. SIMPSON, L. S., and JOLL, C. A. *Endocrinol.*, **22**, 595, 1938.
61. SAPHIR, W., and PARKER, M. L. *J.A.M.A.*, **107**, 1286, 1936.
62. DAVIDSON, C. A., and MOORE, H. D. *Proc. Soc. Exper. & Biol. & Med.*, **35**, 281, 1936.
63. EHRENSTEIN, M., and BRITTON, S. W. *Am. J. Physiol.*, **120**, 230, 1937.
64. CUYLER, W. K., HIRST, D. V., POWERS, J. N., and HAMBLIN, E. C. *J. Clin. Endocrinol.*, **2**, 373, 1942.
65. GUENTHER, A. E. *Endocrinol.*, **5**, 90, 1921.
66. BLANCHARD, C. W. *Phys. Zool.*, **7**, 493, 1934.
67. PERLA, D., and MARMORSTON. *J. Arch. Path.*, **16**, 279, 1933.
68. PERLA, D., and MARMORSTON. "Natural Resistance", p. 475, Little, Brown & Co., Boston, 1941.
69. FOX, C. A., and WHITEHEAD, R. W. *Proc. Soc. Exper. Biol. & Med.*, **32**, 756, 1935.
70. STEINBACH, M. M. *Proc. Soc. Exper. Biol. & Med.*, **27**, 142, 1929.
71. ZWEMER, R. L., and JUNGEBLUT, C. W. *Proc. Soc. Exper. Biol. & Med.*, **32**, 1583, 1935.
72. KEPL, M., CALDWELL, G., and OCHNER, A. *Proc. Soc. Exper. Biol. & Med.*, **52**, 25, 1943.
73. GOLDZIEHER, M. A. "Adrenal Glands in Health and Disease", p. 186, F. A. Davis Co., Philadelphia, 1944.
74. SELYE, H. *Nature*, **138**, 32, 1936.
75. SELYE, H. *Am. J. Physiol.*, **119**, 400, 1937.
76. SELYE, H. *Proc. Soc. Exper. Biol. & Med.*, **38**, 728, 1938.
77. SWINGLE, W. W., HAYS, W. H., REMINGTON, J. W., COLLINGS, W. D., and PARKINS, W. M. *Am. J. Physiol.*, **132**, 249, 1941.
78. GOLDZIEHER, M. A. "Adrenal Glands in Health and Disease", p. 188, F. A. Davis Co., Philadelphia, 1944.
79. ABDERHALDEN, E., PAFFRATH, H., and SICKEL, H. *Pflueg. Arch.*, **206**, 659, 1924.

80. SAKURAI, T. *J. Biochem.*, **6**, 1926.
81. REISS, M., and WINTER, K. A. *Endocrinol.*, **10**, 404, 1932.
82. GOLDZIEHER, M. "Adrenal Glands in Health and Disease", p. 192, F. A. Davis Co., Philadelphia, 1944.
83. ALBERT, S. *Endocrinol.*, **30**, 454, 1942.
84. NOBLE, R. L., and COLLIP, J. B. *Endocrinol.*, **29**, 934 1931.
85. WHITEHEAD, R. *Brit. J. Exp. Path.*, **13**, 200, 1932.
86. FAHR, T. H. *Verhandl. D. Path. Ges.*, **15**, 234, 1912.
87. TEPPERMAN, J., and ENGLE, F. G. Mayo Foundation, 1942.
88. NELSON, D. *Fed. Proc.*, **1**, 62, 1942.
89. HALL, K., and KORENCHEVSKY, K. *J. Physiol.*, **91**, 365, 1938.
90. HOSKINS, R. G. *J.A.M.A.*, **55**, 1725, 1910.
91. SCHMIDT, I. G., and SCHMIDT, L. H. *Endocrinol.*, **23**, 559, 1938.
92. ROSEN, S. H., and MARINE, C. *Proc. Soc. Exper. Biol.*, **41**, 647, 1939.
93. ZWEMER, R. L. *Am. J. Physiol.*, **79**, 658, 1927.
94. THORN, G. W. *J.A.M.A.*, **125**, 10, 1944.
95. KENDAL, E. C. "Glandular Physiology and Therapy", p. 273, Am. Med. Assn., 1942.
96. SARASON, E. L. *Arch. Path.*, **35**, 373, 1943.
97. LAWRENCE, R. D. *Brit. Med. J.*, **1**, 12, 1943.
98. NELSON, W. O., GAUNT, R., and SCHWEIGER, M. *Endocrinol.*, **33**, 325, 1943.
99. HOFFMAN, M. M., KAZMIN, V. E., and BROWNE, J. S. L. *J. Biol. Chem.*, **147**, 259, 1943.
100. SELYE, H., SYLVESTER, HALL, C., and LEBLOND, C. P. *J.A.M.A.*, **124**, 201, 1944.
101. DARROW, D. C., and MILLER, H. C. *J. Clin. Invest.*, **21**, 601, 1942.
102. SELYE, H. *J. Cand. M.A.*, **47**, 515, 1942.
103. CORI, C. F., and WELCH, A. M. *J.A.M.A.*, **116**, 2590, 1941.
104. ARNOW, L. E. *Science*, **86**, 176, 1937.
105. HEIRMAN, P. *Comptes Rend. Soc. Biol.*, **124**, 1250. *ibid.*, **127**, 345, 1938.
106. BACQ, Z. N. *Comptes Rend. Soc. Biol.*, **127**, 341, 1938.
107. CANNON, W. B., and URIDIL, J. E. *Am. J. Physiol.*, **58**, 353, 1921.
108. CANNON, W. B., and ROSENBLIETT, A. "Autonomic Neuro-Effector Systems", MacMillan, New York, 1937.
109. FLORY, H. *J. Physiol.*, **68**, 1, 1927.
110. GOLDZIEHER, M. A. "Adrenals in Health and Disease", p. 51, F. A. Davis Co., Philadelphia, 1944.
111. BROWN, W. C., and WILDER, B. M. *Am. J. Obst. & Gynec.*, **45**, 649, 1943.
112. ESKIN, I. A. *Bull. Exper. Biol. & Med. U.S.S.R.*, **7**, 68, 1944.
113. DE BODO, R. C., BLOCK, H. I., and SLATER, I. *Am. J. Physiol.*, **137**, 671, 1942.
114. GOLDZIEHER, M. A. "Adrenal Glands in Health and Disease", p. 262, F. A. Davis Co., Philadelphia, 1944.

15. *Ibid.*, p. 263.
16. *Ibid.*, p. 267.
17. GROLLMAN, J. A. "Essentials of Endocrinology", Lippincott, Philadelphia, 1943.
18. GOLDZIEHER, M. A. "Adrenals in Health and Disease", p. 208, F. A. Davis Co., Philadelphia, 1944.
19. ELDER, C. A., and KUNNER, A. J. *J. Clin. Endocrinol.*, **3**, 596, 1943.
20. DAVIDSON, C. S. *Proc. Soc. Exper. Biol. & Med.*, **36**, 703, 1937.
21. CLUXTON, H. E., BENNETT, W. A., POWER, M. H., and KEPLER, E. J. *J. Clin. Endocrinol.*, **5**, 61, 1945.
22. PASHKIS, K. E., HERBERT, P. A., RAKOFF, A. E., and CANTAROW, A. J. *J. Clin. Endocrinol.*, **3**, 212, 1943.
23. HALL, G., KELLETT, C. E., and STEPHENSON, G. E. *Lancet*, **1**, 862, 1939.
24. KEPLER, E. J., KENNEDY, R. L. J., DAVIS, A. C., WALTERS, W., and WILDER, R. M. *Mayo Clin.*, **9**, 169, 1934.
25. SUSMAN, W. *Brit. J. Surg.*, **22**, 539, 1935.
26. CROOKE, A. C. *J. Path. & Bact.*, **41**, 339, 1935.
27. SEVERINGHAUS, A. E. *A. Research Nerv. & Ment. Dis. Proc.*, **17**, 69, 1938.
28. SEVERINGHAUS, A. E., and THOMPSON, K. W. *Proc. Soc. Exper. Biol. & Med.*, **40**, 627, 1939.
29. ROCHLIN, M. L. *Problems of Endocrinol.*, U.S.S.R., **2**, 38, 1941.
30. CROOK, A. C., and RUSSEL, D. S. *J. Path. & Bact.*, **40**, 255, 1935.
31. BAUMAN, E. J., and MARINE, D. *Endocrinol.*, **36**, 400, 1945.
32. GOLDZIEHER, M. A. "Adrenal Glands in Health and Disease", p. 273, F. A. Davis Co., Philadelphia, 1944.
33. HOUSSAY, B. A., and MAZZOCCO, A. *Comptes Rend. Soc. Biol.*, **86**, 409, 1922.
34. COPE, C., and MARKS, H. P. *J. Physiol.*, **83**, 137, 1934.
35. SELYE, H. *J.A.M.A.*, **115**, 2246, 1940.
36. COREY, E. L., SILVETTE, H., and BRITTON, S. W. *Am. J. Physiol.*, **125**, 644, 1939.
37. SWANN, H. G. *Physiol. Rev.*, **20**, 483, 1940.
38. KOSTER, H., and KASMAN, L. P. *Arch. Surg.*, **45**, 272, 1942.
39. LANDAU, M. "Die Nebennierenrinde", Jena, 1915.
40. WEGELIN, C. "Henke-Lubarsch. Hdbuch. d. Path. Anat.", VIII, Springer, 1926.
41. RAUTMANN, H. *Mitt. Grenzgeb. Chir. u. Med.*, **28**, 489, 1915.
42. GOLDZIEHER, M. A. "Adrenal Glands in Health and Disease", p. 642, F. A. Davis Co., Philadelphia, 1944.
43. *Ibid.*, p. 644.
44. MARINE, D., and BAUMAN, E. J. *Am. J. Physiol.*, **57**, 136, 1921; *ibid.*, **59**, 353, 1922.
45. SCOTT, W. J. M. *J. Exper. Med.*, **36**, 199, 1922.
46. MARINE, D. *J. Am. Med. Sc.*, **180**, 767, 1930.
47. THADDEA, S. "Die Nebennierenrinde", Leipzig, Thieme, 1936.



148. BARKER, S. B., FAZEKAS, F., and HIMWICH, H. E. *Am. J. Physiol.*, **115**, 415, 1936.
149. SHELLING, D. H. "The Parathyroids", Mosby, St. Louis, 1935.
150. CLAUSEN, H. J. *Endocrinol.*, **27**, 989, 1940.
151. GANES, R. G., and FRIEDGOOD, C. E. *Endocrinol.*, **16**, 305, 1936.
152. BRAM, I. "Exophthalmic Goitre", Mosby, St. Louis, 1936.
153. RICHARDSON, J. A. *Acta. Med. Scand.*, **98**, 581, 1939.
154. OEHME, C. *Klin. Wchnschr.*, **15**, 512, 1935.
155. FREEMAN, W., MELICK, J. M., and MCCLUSKY, D. K. *Am. J. Obst. & Gynec.*, **33**, 618, 1937.
156. KOTZ, J., and KAUFMANN, M. S. *Am. J. Obst. & Gynec.*, **39**, 449, 1940.
157. WAGNER, B. *Zentralbl. f. Gynæk.*, **63**, 432, 1939.
158. LAPIN, J. H., GOLDMAN, S. F., and GOLDMAN, A. *N.Y. State J. Med.*, **43**, 1964, 1943.
159. HART, B. F., MCCONNELL, W. T., and PICKETT, A. N. *Am. J. Obst. & Gynec.*, **48**, 251, 1944.
160. HAMBLIN, E. C., CUYLER, W. K., and BAPTIST, M. *J. Clin. Endocrinol.*, **1**, 763, 1941.
161. ALLEN, R., and BOURNE, G. *Austral. J. Exper. Biol. & Med. Sci.*, **14**, 45, 1936.
162. MIESCHER, K., FISCHER, W. H., and TSCHOPP, E. *Nature*, **142**, 435, 1938.
163. ZUCKERMAN, S. *J. Endocrinol.*, **2**, 311, 1940.
164. HOFF, F. *Deut. Med. Wchnschr.*, **62**, 129, 1936.
165. SALMON, U. J., GEIST, S. H., and SALMON, A. A. *Proc. Soc. Exper. Biol. & Med.*, **47**, 279, 1941.
166. LOPEZ, F. S. *Frankfurt. Ztschr. f. Path.*, **46**, 350, 1934.
167. ELLISON, E. T., and BURCH, G. D. *Endocrinol.*, **20**, 646, 1936.
168. VERZAR, F., and PETER, F. *Pflueg. Arch.*, **206**, 659, 1924.
169. BEZNAK, A. B., and PERJES, J. *Pflueg. Arch.*, **236**, 181, 1935.
170. MOLNER, S., and PETRANYI, J. *Klin. Wchnschr.*, **18**, 1191, 1939.
171. GOLDZIEHER, M. A. "Adrenal Glands in Health and Disease", p. 586, F. A. Davis Co., Philadelphia, 1944.
172. NELSON, A. A. *U.S. Pub. Health Rep.*, **54**, 2250, 1939.
173. UNNA, K., and ANTOPOL, W. *Proc. Soc. Exper. Biol. & Med.*, **43**, 16, 1940.
174. MCCARRISON, R. *Indian J. Med. Res.*, **7**, 260, 283, 1919.
175. HERZENBERG, H. *Beitr. path. Anat.*, **96**, 97, 1935.
176. PACKARD, M., and WESCHLER, H. F. *J. Am. Med. Sci.*, **186**, 66, 1933.
177. HASELTINE, S. L. *J. Am. Inst. Homotherapy*, **36**, 85, 1943.
178. SELYE, H., and SCHENKER, V. *Proc. Soc. Exper. Biol. & Med.*, **39**, 518, 1938.
179. LANGLEY, L. L., and CLARKE, R. W. *Yale J. Biol.*, **14**, 529, 1942.
180. HARTMAN, F. A., and THORN, G. W. *Proc. Soc. Exper. Biol. & Med.*, **28**, 94, 1930.
181. PFIFFNER, K. J., SWINGLE, W. W., and VARSH, J. *Biol. Chem.*, **104**, 701, 1934.

182. TRUSZKOWSKI, R., and DISZYNSKA, J. *Endocrinol.*, **27**, 117, 1940.
183. LONG, C. N. H., KATZIN, B., and FRY, E. G. *Endocrinol.*, **26**, 309, 1940.
184. GRATTAN, J. F., and JENSEN, H. *J. Biol. Chem.*, **135**, 511, 1940.
185. EVERSE, J. W. R., and DE FREMERY, R. P. *Acta. Brev. Neerland*, **2**, 152, 1932.
186. INGLE, D. J. *Endocrinol.*, **26**, 472, 1940.
187. GOLDZIEHER, M. A. "Adrenal Glands in Health and Disease", p. 146, F. A. Davis Co., Philadelphia, 1944.
188. MACBRYDE, C. M., and DE LA BLAZE, F. A. *J. Clin. Endocrinol.*, **4**, 287, 1944.
189. INGLE, D. J. *J. Clin. Endocrinol.*, **4**, 208, 1944.
190. MOORE, T. V. *Psychiat. Quart.*, **16**, 765, 1942.
191. DI MAIO, M., and BIRD, C. E. *New England J. Med.*, **228**, 390, 1943.
192. MCCULLAGH, E. P., LEWIS, L. A., and SHIVELY, F. L., JR. *J. Clin. Endocrinol.*, **3**, 492, 1943.
193. SHIPLEY, R. A. *J. Am. Med. Sc.*, **207**, 19, 1944.
194. BACON, L. C. *Journ. Allergy*, **13**, 48, 1941.
195. WILSON, A. *Lancet*, **1**, 762, 1942.
196. DUNLOP, D. M. *Brit. Med. J.*, **1**, 557, 1943.
197. DUNLOP, D. M. *Practitioner*, **154**, 143, 1945.
198. HENI, F. *Deutsch. Med. Wchnschr.*, **68**, 162, 1942; *ibid.*, **68**, 318, 1942.
199. BARTELS, E. C. *Lahey. Chem. Bull.*, **3**, 173, 1943.
200. GOLDZIEHER, M. A. "Adrenal Glands in Health and Disease", p. 327, F. A. Davis Co., Philadelphia, 1944.
201. *Ibid.*, p. 316.
202. GOLDZIEHER, M. A., and BARISHAW, S. B. *Endocrinol.*, **21**, 394, 1937.
203. KATZ, F., and MAINGER, F. *Brit. Med. J.*, **1**, 617, 1941.
204. AUSLANDER, E. M. *Nov. Chir. Arch.*, **42**, 375, 1939.
205. BROSTER, L. R. *Proc. Roy. Soc. Med.*, **40**, 35, 1946.
206. SIROTA, H. A. *J. Clin. Endocrinol.*, **3**, 141, 1943.
207. WEICHARD, U. *Strahlen Therapie*, **71**, 127, 1942.
208. KOHLER, V., and FLECKENSTEIN, A. *Deutsch. Med. Wchnschr.*, **68**, 476, 1942.
209. JACOBS, L. *Arch. Ped.*, **60**, 313, 1943.
210. SCUDDER, J., and ELLIOTT, R. H. E., JR. *South. Med. & Surg.*, **104**, 651, 1942.
211. VENNING, E. H., KAZMIN, V. E., and BELL, J. C. *Endocrinol.*, **38**, 79, 1946.
212. DORFMAN, K. J., SHIPLEY, R. A., ROSS, E., SCHILLER, S., and HORWITT, E. *Endocrinol.*, **38**, 189, 1946.
213. LEWIS, L. A., and PAGE, I. H. *J. Lab. & Clin. Med.*, **31** (12), 1325, 1946.
214. VENNING, E. H., and BROWNE, J. S. L. *J. Clin. Endocrinol.*, **7**, 79, 1947.
215. SPENCE, H. M., and THOMPSON, F. G., JR. *New England J. Med.*, **236**, 13, 1947.
216. SALTER, W. T., CAHEN, R. L., and SAPPINGTON, T. S. *J. Clin. Endocrinol.*, **6**, 52, 1946.
217. HOUSSAY, B. A. *Rev. As. med. argent.*, **60**, 83, 1946.

218. SCOWEN, E. F., and WARREN, F. L. *Proc. Roy. Soc. Med.*, **40**, 40, 1946.
219. VENNING, E. H. *Endocrinol.*, **39**, 203, 1946.
220. MCFADZEAN, A. J. S. *Lancet*, **251**, 940, 1946.
221. SHIPLEY, R. A., and DORFMAN, R. G. *J. Lab. & Clin. Med.*, **31** (4), 481, 1946.
222. SELYE, H. *J. Clin. Endocrinol.*, **6**, 117, 1946.
223. SELYE, H. *McGill News*, Montreal, 1937.
224. SELYE, H. *Endocrinol.*, **21**, 169, 1937.
225. DALTON, A. J., DOSNE, C., and SELYE, H. *Anat. Rec. Suppl.*, **76**, 85, 1940.
226. DALTON, A. J., MITCHELL, E. R., JONES, B. F., and PETERS, V. B. *J. Nat. Cancer Inst.*, **4**, 527, 1944.
227. ZWEMER, R. L. *Am. J. Path.*, **12**, 107, 1936.
228. LEVIN, L. *Endocrinol.*, **37**, 34, 1945.
229. SAYERS, G., SAYERS, M. A., FRY, E. G., WHITE, A., and LONG, C.N.H. *Yale J. Biol. & Med.*, **16**, 361, 1943.
230. SELYE, H. *Brit. J. Exper. Path.*, **17**, 234, 1936.
231. UNGAR, G. *J. Physiol.*, **103**, 333, 1944.
232. BROWNE, J. S. L., and JOSIAH MACY, JR. Foundation Conference on Metabolic Aspects of Convalescence including Bone and Wound Healing, Fourth Meet., June 1943.
233. GLENDENING, M. B., WINTER, H. A., WILLIAMS, H. H., ABBOT, W. E., HIRSCHENFELD, J. W., and HELLER, C. G. Twenty-Seventh Ann. Meet. of Assn. for Study of Internal Secretions, Chicago, June 1944.
234. VENNING, E. H., and BROWNE, J. S. L. *Federation Proc.*, **4**, 108, 1945.
235. VENNING, E. H., HOFFMAN, M. M., and BROWNE, J. S. L. Twenty-Seventh Ann. Meet. of Assn. for Study of Internal Secretions, Chicago, June 1944.
236. WEIL, P. G., and BROWNE, J. S. L. *Science*, **90**, 445, 1939.
237. WEIL, P. G., and BROWNE, J. S. L. *Proc. Canad. Physiol. Soc. Kingston*, 1939.
238. WHITE, A., and DOUGHERTY, T. F. *Endocrinol.*, **36**, 207, 1945.
239. DOUGHERTY, T. F., CHASE, J. H., and WHITE, A. *Proc. Soc. Exper. Biol. & Med.*, **57**, 295, 1944.
240. HARRIS, T. N., GRIMM, E., MERTENS, E., and EHRLICH, W. E. *J. Exper. Med.*, **81**, 73, 1945.
241. DOUGHERTY, T. F., WHITE, A., and CHASE, J. H. *Proc. Soc. Exper. Biol. & Med.*, **56**, 28, 1944.
242. CANNON, P. R. *J. Immunol.*, **44**, 107, 1942.
243. DOUGHERTY, T. F., CHASE, J. H., and WHITE, A. *Proc. Soc. Exper. Biol. & Med.*, **58**, 135, 1945.
244. COURRIER, R. *Ann. d'Endocrinologie*, **1**, 533, 1939-40.
245. ALVAREZ, E., and FUENZALIDA, F. *Proc. Soc. Exper. Biol. & Med.*, **62**, 132, 1946.
246. SELYE, H., BELAND, E., and SILVESTER, O. *Exper. Med. & Surg.*, **2**, 224, 1944.



- 247. MANDL, F. *Wien. Klin. Wchnschr. Vienna*, **38**, 11, 1947.
- 248. SCHNEIDER, H. O. *Northwest Med.*, **46**, 216, 1947.
- 249. GUTMAN, D. *Brit. Med. J.*, 4503, 563, 1947.
- 250. SPALDING, J. M. K. *Brit. Med. J.*, 4503, 564, 1947.
- 251. ESPERSEN, T., and DAHL-IVERSON, E. *Acta. Chir. Scand.*, **94**, 271, 1946.
- 252. DAVIES, A. W., and MOORE, T. *Biochem. J.*, **28**, 288, 1934.
- 253. POPPER, H. *J. Mount Sinai Hosp.*, **7**, 119, 1940; *Am. J. Physiol.*, **99**, 467, 1941.
- 254. SZENT-GYÖRGYI, A. *Science*, **72**, 125, 1930.
- 255. MORAWITZ, P. *Klin. Wchnschr.*, **13**, 324, 1934.
- 256. ROTHMAN, S. *J. Invest. Dermatol.*, **5**, 67, 1942.
- 257. DORFMAN, R. I., ROSS, E., and SHIPLEY, R. A. *Endocrinol.*, **38**, 178, 1946.

## THE ISLET SYSTEM OF THE PANCREAS

THE pancreas as a gland of internal secretion will be discussed here mainly in its relationship with other glands and in connection with the rôle of insulin in the regulation of blood sugar.

The islet system consists of an aggregation of spheroidal cells arranged in irregular columns in the connective tissue between the acini of the interlobular ducts. Some of the islets are connected with the pancreatic ducts.

The cells of the islets are of two general types, called A and B cells. The A cells have large vesicular nuclei and large acidophilic granules; the B cells have smaller nuclei and basophilic granules. Experiments have shown that the B cells are concerned with the production of insulin. This, however, does not signify that they are distinct entities, but more probably that they represent merely a particular stage of activity.

## CONTROL OF INSULIN SECRETION

The factors which control insulin secretion are, so far, unknown. Investigation of the problem is difficult, for there is as yet no method for determining the insulin concentration of the blood. Opinions differ as to whether the nervous system or the composition of the blood is the stimulating factor.

Glucose hyperglycaemia is held to cause increased secretion of insulin. There is evidence that the concentration of sugar in the blood passing through the pancreas influences the rate of insulin secretion [1]. Stimulation of the vagus produces increased function of the islets of Langerhans.

The production of diabetes in the rabbit by puncture of the floor of the fourth ventricle, as first demonstrated by Claude Bernard, indicated that the control of blood sugar was situated in the brain. It is suggested that this regulatory centre is localized in the hypothalamus and midbrain [2]. The recent hypothesis of an insulotrophic principle in the pituitary [3] is based on the hypoglycaemic effect obtained with pituitary extract in two patients with excessively functioning islet tissue due to a pancreatic islet-cell tumour. The same pituitary extract injected into normal dogs produced hyperglycaemia. Evaluation of these results requires further studies in the experimental animal. The most recent conception of the control of insulin secretion under normal conditions, however, is that it is more or less constant and that the blood sugar level is regulated by the liver [4].

In the light of recent evidence the control of blood sugar seems a more complex function than has hitherto been believed—several glands, i.e. the anterior pituitary, thyroid, adrenal cortex and adrenal medulla, being connected with blood-sugar regulation [5] (see Fig. 5). It has therefore become necessary to abandon the conception that hypoglycaemia and hyperglycaemia are expressions of hyperinsulinism and hypoinsulinism respectively. Every change in the blood-sugar level must be regarded as a sign of disturbed function of one or more glands, or of the correlation of the endocrine system as the whole.

#### PHYSIOLOGY

Injection of insulin lowers the blood-sugar level. Experiments *in vitro* have shown that insulin has no direct action on blood glucose, apparently eliciting its action directly on the tissues, in which it promotes some chemical change termed by McLeod "Vacuum for Glucose".

Insulin in low concentration seems to influence carbohydrate metabolism by inhibiting liver glycogenolysis and in this way increasing glycogen storage [6]. It further facilitates and perhaps controls the formation of muscle glycogen from blood sugar [7], inhibits gluconeogenesis (formation of glucose from protein and fats), and facilitates the peripheral oxidation of glucose [8].

Soskin [6] demonstrated that, in the pancreatectomized dog, increased administration of glucose with a constant supply of insulin could not produce hyperglycaemia; though this did occur on increased administration of glucose to the hepatectomized dog with a normal pancreas. It thus appears that provided sufficient insulin is produced by the islet system, an additional supply is not required to maintain a normal glucose tolerance curve after administration of glucose. On the basis of this experiment it may be concluded that the normal glucose tolerance curve does not result from the stimulation of increased insulin secretion after administration of glucose as hitherto believed, but on the other factors regulating the blood-sugar level.

Provided that the functions of all other factors concerned in carbohydrate metabolism are normal, the requirements of insulin for the maintenance of a normal blood-sugar level are surprisingly small. Goldner and Clark [9] showed, in two pancreatectomized patients, that only 30–50 units of insulin were required daily in order to maintain a normal blood-sugar level with a glucose intake of 150–200 grams daily.



## The Liver

The liver acts as a homeostatic mechanism in the regulation of blood sugar. Its function is controlled by a number of endocrine glands, the blood-sugar level, the insulin secretion and the carbohydrate intake.

An experimental study of this mechanism was made by Soskin and others [10], who applied the dextrose tolerance test with a quantitative study of the amounts of sugar which entered and left the liver per unit of time. Immediately after administration of dextrose the liver ceased its output of sugar and started to store it in large quantities. This was followed by a period of sugar retention during which the liver neither took in nor put out sugar. The end of this period was characterized by a lower blood-sugar level than before the test. After this period of inhibition the liver began to release its usual supply of sugar to the blood. Thus, according to Soskin [5], the normal blood-sugar level depends on the amount of sugar being supplied by the liver and the amount of sugar being withdrawn from the blood by the tissues.

In diabetes the storage of liver glycogen is impaired; glucose leaves the liver in larger than the normal amounts, being derived by glyconeogenesis from proteins and fats. Increased protein catabolism takes place, resulting in increased nitrogen excretion. The depletion of body fat causes increased lipemia and an increase of acetone bodies. Insulin protects the body fat depots and, by inhibiting the oxidation of fats, exerts an antiketolytic action.

Soskin described the aetiology of the two types of clinical diabetes mellitus, the insulin sensitive (juvenile or unstable) and the insulin insensitive (adult or stable). The former type is characterized by damage of the islet system without liver impairment, whereas the insulin insensitive type is the one with impaired liver function.

The high fat and starvation regimes formerly used in the treatment of diabetes mellitus resulted in fatty infiltration of the liver with impairment of its function. The diabetic condition was in fact improved, but only at the cost of a damaged liver and the well-being of the patient.

Soskin recommends his intravenous dextrose tolerance test for liver dysfunction. In this test  $\frac{1}{3}$  gram of dextrose per kilogram body-weight is injected intravenously before breakfast in a 50 per cent aqueous solution within a period of 3-5 minutes. Blood samples are taken before the sugar is administered and again a half, one and two hours later. The blood-sugar level of normal subjects takes less than 60 minutes to return to the pre-injection level. In even mild diabetics

it takes over 120 minutes; and in patients with impaired liver function it takes less than 120 minutes. In 25 per cent of patients with impaired liver function the blood sugar returned to the pre-injection level in less than 60 minutes.

### **The Anterior Pituitary Gland**

There is no generally accepted proof that the pituitary excretes either a pancreatrophic or a diabetogenic hormone.

Hypopituitarism is characterized by a low blood-sugar level, and the hypophysectomized animal shows a marked sensitivity to insulin [11]. Sugar tolerance tests in a man of 36, in whom total hypophysectomy had been done 5 years previously, seemed to show that total removal of the pituitary lowers the fasting blood sugar to a hypoglycaemic level without producing shock and gives a delayed sugar tolerance curve which at times may form a plateau [12].

Hyperpituitarism, on the other hand, is often associated with hyperglycaemia or diabetes; and it seems that anterior pituitary extracts raise the blood sugar, having apparently an action opposite to that of insulin. Pullen and Sodeman [13] reported a case of diabetes mellitus associated with hirsutism and insulin resistance. This patient received 260 units of crystalline insulin daily without any particular effect upon the carbohydrate metabolism; but this definitely improved under X-ray therapy to the pituitary gland.

Dubrovsky [14] considered the symptom triad hyperglycaemia, glycosuria and reduction in sugar tolerance not as the disease entity diabetes mellitus but as a syndrome found in a diversity of pituitary disorders. When this diabetic triad occurs in patients at about the sixth decade of life it indicates lowering of all body functions including that of metabolizing food. In women at 40–50 years the aetiological factor may be the particular activity of the anterior pituitary initiated by menopausal ovarian failure.

Bovine pituitary implants into 12 patients suffering from impaired pituitary function were followed in all cases by mild to sharp rises in blood sugar [15]. Cats were made diabetic by injections of anterior pituitary extracts, and if the diabetic condition was allowed to exist for more than 3 months without treatment, it resulted in atrophy of the islets of Langerhans and permanent diabetes. Treatment with insulin or phlorhizin within 3 months results in improvement of diabetes and restoration of the islets [16].

Soskin and others [17] demonstrated that the Staub-Traugott effect (repeated dextrose tolerance tests following in close succession, showing in the normal animal a consecutive lowering of the blood-

sugar curve up to the fourth test) occurs only in the presence of a normal functioning anterior pituitary gland. In the absence of the hypophysis the first curve is the lowest. The administration of anterior pituitary extract raises the level of all tests without restoring the Staub-Traugott phenomenon. Soskin [17], in the light of these paradoxical findings, explains the Staub-Traugott effect as being due to a progressive depression in the activity of the pituitary gland brought about by repeated or prolonged exposure to hyperglycaemic levels. No evidence is given that hyperglycaemia depresses pituitary activity. It is reasonable to assume that the Staub-Traugott effect is produced by progressive increase of sugar retention in the liver followed by subsequent failure, as demonstrated in Soskin's use of the dextrose tolerance test with an estimate of the amounts of sugar entering and leaving the liver per unit of time [10]. Failure of the liver to retain sugar in the hypophysectomized animal is probably caused by secondary adrenal cortical deficiency. The further rise of blood sugar following injections of pituitary extracts is difficult to explain and calls for investigation. Recent experiments have shown that pituitary extract is not diabetogenic in the adrenalectomized animal [18].

### **Pituitrin**

Subcutaneous injection of pituitrin 20 units neutralizes the action of 15 units of insulin also given subcutaneously [19].

### **The Adrenal Cortex**

Hyperglycaemia and diabetes are frequently associated with hyperinterrenalism and sometimes become the dominating symptoms, as for example in Achar-Tiers syndrome. Hypoglycaemia, on the other hand, is associated with all hypointerrenal syndromes, including Addison's disease.

Hyperfunction of the adrenal cortex causes increased gluconeogenesis, and if this becomes excessive, the liver excretes abnormal amounts into the blood. Hyperglycaemia is caused in this manner and finally results in exhaustion of the islet system.

Increased lipemia is due to an increase of gluconeogenesis and points to the adrenal cortex and liver as the pathogenic factors in diabetes. Insulin in large doses fails significantly to lower the increased lipemia in uncontrolled diabetic subjects [20]. According to Goldzieher [21] the massive lipemia in diabetic coma, caused by mobilization of fat from its depots, is an expression of acute cortical insufficiency. The sudden hyperpyrexia which occasionally develops



in the terminal stage of diabetes, as a result of dehydration [22], is probably due to adrenal cortical failure.

Janes and others [23] induced diabetes in rats of the Long-Evans strain which showed, after adrenalectomy, reduction or complete disappearance of diabetic symptoms.

Insulin exerts a stimulating action on the adrenal cortex, hypertrophy occurring after its prolonged administration (see Chapter V).

### **Adrenal Medulla**

Adrenalin, whether given subcutaneously or intravenously, markedly antagonizes the action of insulin. Increased activity of the medulla is usually associated with hyperglycaemia, whereas insulin lowers the blood-sugar level by increased oxidation of sugar and inhibition to glycogenolysis.

The antagonism of insulin to adrenalin concerns only carbohydrate metabolism, since the vasoconstrictor effect of adrenalin is not inhibited by insulin.

### **Thyroid Gland**

Hyperglycaemia is sometimes associated with hyperthyroidism, and thyroidectomy frequently improves diabetes.

Excessive thyroid feeding depletes the liver and muscle glycogen, exerting its action apparently by increasing the effect of adrenalin.

### **Gonads**

Before the introduction of insulin for treatment of diabetes, stunting of growth and genital retardation were common manifestations in juvenile diabetes. In the adult, menstrual disturbances and sterility are common in the female, as are impaired spermatogenesis with impotence in the male.

The incidence of diabetes is highest during or shortly after the menopause.

Cramer [55] observed, in diabetic patients, a correlation between menstruation and diminution in carbohydrate tolerance, which sometimes was severe enough to precipitate diabetic acidosis. Even in non-diabetics, sugar tolerance has been found impaired in the menstrual period.

Much controversy exists as to the influence of oestrogen on the blood-sugar level [24, 25, 26, 27, 28]. Ganen [29] obtained improvement in 50 per cent of diabetic patients by daily intramuscular injections of oestrone 10,000–50,000 I.U. Others report an increase of

blood sugar and liver glycogen [30, 31, 32, 33] or no effect at all [34, 35].

These contradictory results are difficult to explain. It is possible that the corticomimetic effect of oestrogen plays some part in determining these different results which may further be influenced by

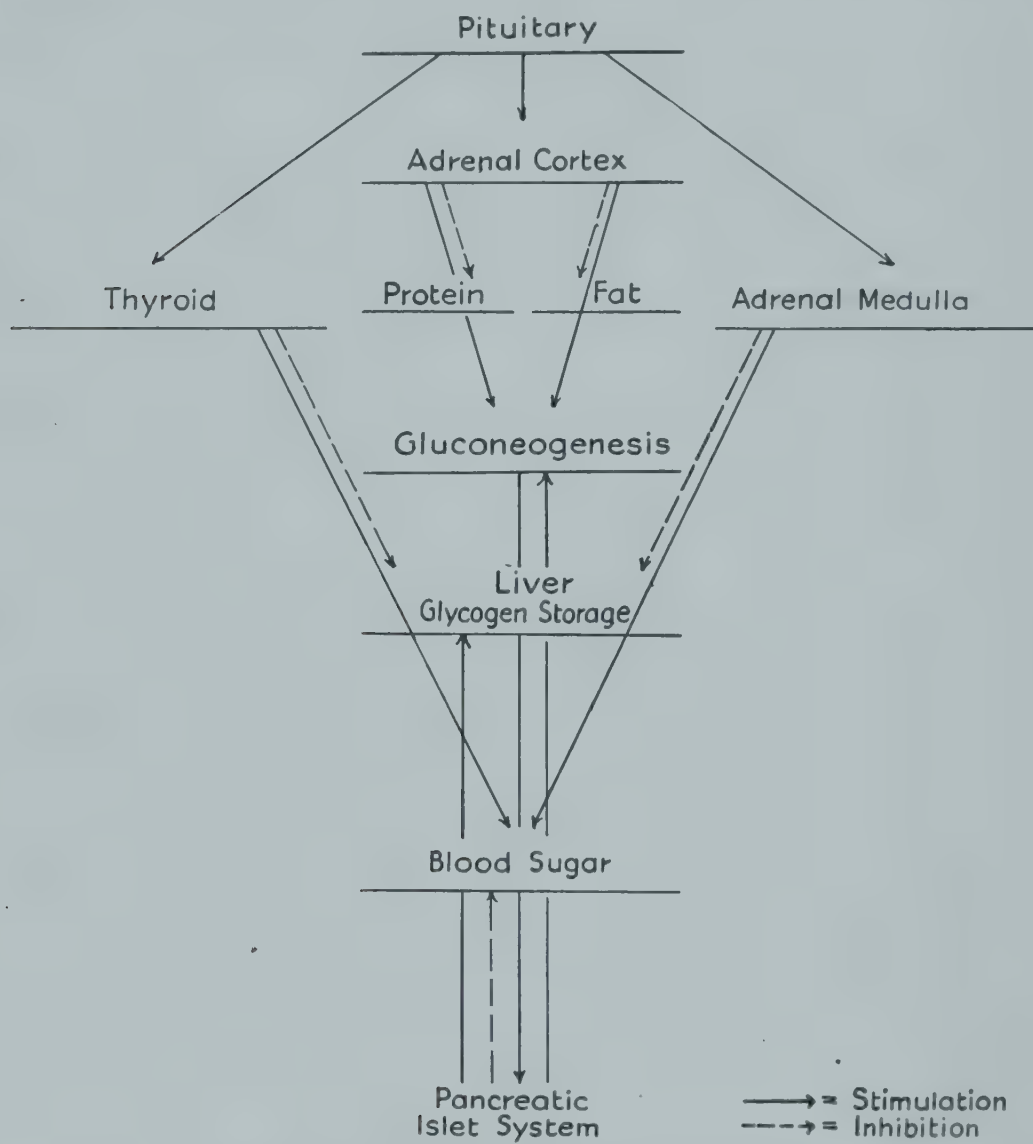


FIG. 5.—THE ROLE OF THE ENDOCRINE GLANDS IN BLOOD SUGAR REGULATION

the dosage of oestrogen and the duration of treatment. Ingle [36], however, demonstrated that the diabetogenic activity of diethylstilboestrol was not mediated through the anterior pituitary or through the adrenal and pituitary glands together. In adrenalectomized, hypophysectomized, partially-depancreatized male rats, kept on a sub-diabetogenic regime of adrenal cortical and anterior pituitary

extracts, the administration of diethylstilboestrol caused hyperglycaemia and glycosuria, though all the test animals were non-diabetic during control periods.

### **Growth Hormone**

Administration of growth hormone and oestrone to the depancreatized animal decreases the insulin requirements [37]. Anderson and Long recently demonstrated that purified growth hormone prevents the insulin secretion which would be expected to occur with an elevated blood-sugar. Rat pituitary extract inhibits insulin secretion in the same way as the growth hormone [56]. Insulin hypoglycaemia and shock in the male rat are associated with inhibition of growth or definite loss of weight, increased sodium and chloride excretion, followed by compensatory retention and increased excretion of non-protein nitrogen [38].

### **Alloxan**

Alloxan, a simple combination product of urea and mesoxalic acid, is commonly prepared in the laboratory by the oxidation of uric acid with strong oxidizing agents such as dilute nitric acid. Dunn and others [39, 40, 41] suggest that alloxan stimulates islet activity, the cells finally failing and undergoing necrosis because they have been overdriven. They advance the hypothesis that, under physiological conditions, alloxan may be formed in the body and act as a muscle hormone in regulating islet activity. They refer to it as a possible cause of a disturbance of the islet system which eventually may develop into diabetes mellitus.

### **Vitamin B**

There is considerable evidence on the participation of vitamin B in carbohydrate metabolism. Abderhalden contends that vitamin B<sub>1</sub> is essential for the intermediate metabolism and utilization of carbohydrates. Its requirements increase in relation to the amount of carbohydrate in the food. If the latter is excessive, B<sub>1</sub> avitaminosis may develop in spite of normal supplies.

Two of the intermediate products of carbohydrate metabolism are lactic acid and pyruvic acid. The brains of pigeons suffering from avitaminosis-B<sub>1</sub> show increased lactic acid in their lower parts [42]. Further investigations have shown that the B<sub>1</sub>-deficient cells of the pigeon brain, respiring in the presence of added lactic acid, produce an increased amount of pyruvic acid; but that this level is reduced if vitamin B<sub>1</sub> is added to the diet. The fall in pyruvic acid is attributed



to the fact that vitamin B<sub>1</sub> is phosphorylated in the tissues to form cocarboxylase, which acts as a co-enzyme assisting pyruvic acid carboxylase or its oxydase in breaking down pyruvic acid.

There has been much controversy on the clinical value of vitamin B<sub>1</sub> in the treatment of diabetes. Reports of its hypoglycaemic action are plentiful, but so are negative results. It seems that further investigations, based on present knowledge of carbohydrate metabolism, may establish the rôle of vitamin B<sub>1</sub>, possibly as a coadjunct, in the treatment of diabetes (see Chapter XIV).

#### STANDARDIZATION

The International Unit adopted for insulin is equivalent to 0.045 mg. of a standard preparation of dried hydrochloride, which is preserved in the National Institute of Medical Research in London. Each milligram of this standard preparation contains 22 Units as originally defined by the Insulin Committee of the University of Toronto.

One unit is the amount of extract which, in a healthy rabbit of about 2 kilograms weight, after 24 hours' fasting, depresses blood-sugar to 45 mg. per 100 c.c. This is the level at which convulsions occur.

All forms of commercial insulin are dispensed in 5 or 10 c.c. rubber-capped vials in various concentrates designated "U-10", "U-20", "U-40", and "U-80" and so forth, to indicate the number of units contained in each c.c. The manufacture of the concentrates "U-10", "U-20", and "U-100" has been discontinued in the U.S.A.

#### PREPARATIONS FOR CLINICAL USE AND ADMINISTRATION

Unmodified or regular insulin.

Crystalline soluble insulin.

Protamine zinc insulin.

Modified protamine zinc insulin.

Globin zinc insulin.

#### Unmodified or Regular Insulin

The process by which insulin is extracted from the pancreas yields a purified extract which is not the actual hormone usually active in the intact body but a more rapidly acting fraction derived from the hormone itself. From 1922 until 1936 insulin was available commercially in only one form, an aqueous solution, insulin hydrochloride, which is generally known as "unmodified" or "regular" insulin.

It is obtainable in rubber-capped vials in three different concentrations—"U-40", "U-80" and "U-100"—which means that 1 c.c.

contains 40, 80 or 100 "units" of insulin. The smaller concentrations of "U-10" and "U-20" have been discontinued by most manufacturers.

### **Crystalline Soluble Insulin**

"Zinc insulin crystals in solution" is a solution of crystalline insulin to which minute amounts of zinc have been added. This is now commercially available and is rapidly replacing unmodified insulin in clinical practice. It is also referred to as "crystalline insulin" or "crystalline soluble insulin". It is superior to unmodified insulin by its greater purity, lower protein content and reduced tendency to provoke allergic reactions. When it is injected subcutaneously the hypoglycaemic effect appears in about half an hour with its greatest effect in from 3 to 5 hours and a return of the blood-sugar to the starting level in from 6 to 8 hours. The size of the dose influences the degree and duration of the effect. Injected intravenously it produces a reduction in the blood-sugar level within a few minutes. The greatest hypoglycaemic effect is noted in from 2 to 4 hours, and, depending on the size of the dose, the effect wears off in from 4 to 6 hours. The preparation is available in "U-40" and "U-80" concentrations.

### **Protamine Zinc Insulin**

This is a preparation of a precipitated insulin which dissolves slowly at the reaction of subcutaneous fluids. Its slow absorption causes a prolonged but retarded effect. The blood-sugar begins to fall within 3-6 hours and the greatest effect is obtained between 16 and 20 hours after the injection. The duration of hypoglycaemia is more than 24 hours. One single injection of PZI may replace two or more daily injections of soluble insulin. If a morning injection of PZI does not control the rise of blood-sugar after breakfast, an injection of soluble insulin is required in addition, preferably in a separate syringe, before the injection of PZI.

Protamine zinc insulin is available in 10-c.c. vials containing 40 or 80 units of insulin per c.c., in addition to 0.3-0.5 mg. of protamine, from 0.08 to 0.1 mg. of zinc, disodium acid phosphate (as a buffer), glycerine (to maintain isotonicity) and cresol or phenol as a preservative.

### **Modified Protamine Zinc Insulin**

A modification of protamine zinc insulin containing 0.5 mg. of protamine per 100 units (instead of the standard 1.2 mg.) has 25 per

cent of the insulin content in soluble, quickly absorbed form, while 75 per cent is in the precipitate and is slowly absorbed over a period exceeding 24 hours.

In an extensive clinical trial MacBride and Roberts [43, 57] found that the modified protamine zinc insulin had the advantage of acting in smaller doses than its components given separately in a 3 : 1 ratio, and that only one injection was required.

### **Globin Zinc Insulin**

The action of globin zinc insulin seems to be more rapid (30–60 minutes) than that of protamine zinc insulin, but both drugs have the same maximal effect in 8–15 hours. Globin zinc insulin is effective, however, for only about 20 hours [58].

According to Levitt and Schaus [44] globin insulin exerts its maximum effect in about 8 hours and is completely used up in 18 hours. Hypoglycaemic reactions, when present, usually appear in the afternoon and can be obviated by proper arrangement of the diet.

Reiner and others [45] studied the absorption rates of insulin, protamine zinc insulin and globin insulin by introducing radio-active iodine into the insulin molecule and then measuring the decrease of radio-activity at the site of injection after subcutaneous administration to rabbits. It was found that protamine zinc insulin took about 15 times as long as insulin, and globin zinc insulin 3 times as long, to reach the level at which 40 per cent of the radio-activity had disappeared.

MacBride and Reiss [46], in a comparison between modified zinc insulin (protamine zinc insulin 75 per cent, soluble insulin 25 per cent) and globin zinc insulin, showed that globin zinc insulin in no case regulated the blood-sugar more effectively than modified protamine zinc insulin. In many cases, however, protamine zinc insulin was the more effective. Malins [47] found that the greatest drawback to the use of globin insulin was the frequency of hypoglycaemia. Nevertheless, it has a definite place in the treatment of mild or moderately severe cases requiring less than 30 units daily.

### **Di-insulin**

In Denmark, Hey [63] has reported success with an insulin mixture, "Di-insulin" (DI), which exerts a rapid and prolonged action. This preparation consists of equal parts of soluble insulin (SI) and of a new derivative, phenyl-ureido-insulin (iso-insulin), discovered by Hallas-Møller [64] in 1944. In contrast to the protamine insulins, iso-insulin has the desirable property of being miscible with SI without altering



the proportions of the two components. Its action on the blood-sugar is similar to that of protamine insulin without zinc—i.e. it has a slow initial effect, a morning dose not controlling the blood-sugar for some hours after breakfast but acting satisfactorily throughout the remainder of the day and early part of the night. Its effect lasts 16–24 hours after injection [64]. The mixture DI is a clear solution containing 40 units per c.c. It is manufactured by Novo Terapeutisk Laboratorium, Copenhagen.

The protracted effect of the iso-insulin component was demonstrable in the normals and in diabetics requiring less than 50 units of insulin. In the latter a single injection of di-insulin controlled the blood-sugar throughout the 24 hours; but in those requiring 50 units or more one injection of di-insulin was unsatisfactory, producing hypoglycaemia 3–8 hours after injection, and not controlling the blood-sugar in the late evening and during the night [65].

### **Lipocaic**

The name “Lipocaic” was given to an extract of beef pancreas which prevented the fat infiltration and degeneration that commonly developed in the livers of depancreatized dogs which were kept alive with insulin but did not receive fresh pancreas. This substance, extracted from the pancreas by Dragstadt [59, 60], is not identical with choline or lecithin and probably represents a specific secretion, distinct from insulin, concerned with normal transport and utilization of fat.

### **ADMINISTRATION**

Insulin is usually administered subcutaneously. In cases of emergency soluble preparations may be injected intravenously.

### **CLINICAL APPLICATION AND APPROXIMATE DOSAGE**

#### **Diabetes Mellitus**

Uncomplicated Diabetes on a standard dietary regime.

Insulin: (soluble) 1 unit for every  $1-1\frac{1}{3}$  gram of urinary glucose appearing in the 24-hour specimen.

This formula is subject to adjustment to the individual needs of the patient.

Protamine zinc insulin: This is usually given in combination with soluble insulin in a ratio of 1 : 2 or 1 : 3 to replace two or more injections of soluble insulin.

The initial dosage in mild diabetes may be approximately PZI 6 units and soluble insulin 12 units. For moderately severe cases, PZI 24 units and soluble insulin 12 units is advocated [61]. The initial dose should rarely exceed 60 units.

Globin insulin: Since it combines both prompt and prolonged actions, it is often effective as a single dose in cases of moderate severity [61].

### **Schizophrenia (Shock Treatment)**

A high proportion of recoveries have been attributed to the use of insulin in the hypoglycaemic shock treatment of schizophrenia. Polatin and Spotnitz [48] believe that the more rapidly the hypoglycaemic shock is produced the better the therapeutic results.

### **Schizophrenia (Ambulatory Treatment)**

Insulin in doses sufficient to elicit a physiological response, but not high enough to produce coma, produced improvement in 36 of 44 schizophrenic patients [48]. Insulin shock therapy also increases the recovery rate in puerperal psychosis of the schizophrenic type [49]. According to Baker [50] irreversible changes in nervous tissues may be induced by long continued hypoglycaemia, and cases of cerebral damage have been reported following insulin treatment of schizophrenia. These changes, however, may be the result of post-mortem autolysis.

### **Miscellaneous Uses**

Insulin has also been used in malnutrition, drug addiction, recurrent vomiting, acne [51, 52] and dysmenorrhea [53, 54]. The results, however, have been equivocal and its use is not advocated at this stage. Insulin shock treatment has recently been recommended for allergic bronchial asthma [62]. Soluble insulin 20 units were injected hypodermically or intravenously for the first shock and increased by 5 or 10 units for the subsequent treatment. "Shocks" were usually repeated twice weekly and sometimes in the early stages 3 times a week. Very good results are reported from this procedure.

### **BIBLIOGRAPHY**

1. HOUSSAY, B. A. *Am. J. M. Sc.*, **193**, 58, 1937.
2. MCLEOD, J. J. R. *Bull. Johns Hopkins Hosp.*, **54**, 79, 1934.
3. CONN, J. W., and LAWRENCE, L. *J. Clin. Endocrinol.*, **5**, 247, 1945.

4. HIMSWORTH, H. P. *Lancet*, **2**, 165, 188, 171, 1939.
5. SOSKIN, S. *J. Clin. Endocrinol.*, **4**, 75, 1944.
6. SOSKIN, S., ALLVISE, M. D., and COHN, D. J. *Am. J. Physiol.*, **109**, 155, 1934.
7. CAMERON. "Recent Advances in Endocrinology", p. 80, Churchill, London, 1945.
8. HAMBLIN, E. C. "Endocrinology of Women", Chas. C. Thomas, Springfield, Illinois, 1945.
9. GOLDNER, M. G., and CLARKE, D. E. *J. Clin. Endocrinol.*, **4**, 194, 1944.
10. SOSKIN, S., ESSEX, H. G., HERRICK, J. F., and MANN, F. C. *Am. J. Physiol.*, **124**, 558, 1939.
11. HOUSSAY, B. A. *New England J. Med.*, **214**, 971, 1936.
12. HART, J. F., and MAGIDAY, M. *Acta. Int. Med.*, **68**, 893, 1941.
13. PULLEN, R. L., and SODEMAN, W. A. *J. Clin. Endocrinol.*, **3**, 345, 1943.
14. DUBOVSKY, B. *Diabetes Mellitus, Med. Rec.*, **156**, 358, 1943.
15. DE CASTILLO, E. *Reforzo J. Mebrives and Luchette Santos E. Medicina Buenos Aires*, **3**, 166, 1943.
16. LUKENS, F. D. W., DOHAN, F. C., and WOLCOTT, N. W. *Endocrinol.*, **32**, 475, 1943.
17. SOSKIN, S., MIROKY, I. A., ZIMMERMANN, L. M., and HELLER, R. E. *Am. J. Physiol.*, **116**, 148, 1936.
18. GOLDZIEHER, M. A. "Adrenal Glands in Health and Disease", p. 653, F. A. Davis Co., Philadelphia, 1944.
19. WISHNOFSKY, M., KANE, A. P., and BYRON, C. S. *J. Am. Med. Sc.*, **208**, 361, 1944.
20. CAPLAN, A., ENKERMANN, C., and CHAIKOFF, Q. L. *Endocrinol.*, **32**, 247, 1943.
21. GOLDZIEHER, M. A. "Adrenal Glands in Health and Disease", p. 657, F. A. Davis Co., Philadelphia, 1944.
22. HIMWICH, H. G., FAZEKAS, J. F., NAHUM, L. H., DUBOIS, D., GREENBURG, L., and GILMAN, A. *Am. J. Physiol.*, **110**, 19, 1934.
23. JANES, R. G., and FRIEDGOOD, C. E. *Endocrinol.*, **36**, 62, 1945.
24. HENLEY, U. *New Zealand M.J.*, **39**, 308, 1940.
25. BARNES, B. O., REGAN, J. T., and NELSON, W. O. *J.A.M.A.*, **101**, 926, 1933.
26. KURZROK, R. "The Endocrines in Obstetrics and Gynaecology", Williams & Wilkins, Baltimore, 1938.
27. SPIEGELMAN, A. R. *Proc. Soc. Exper. Biol. & Med.*, **43**, 307, 1940.
28. WILDER, R. M. "Hyper-Insulinism; Definition and Diagnosis", *Mississippi Doctor*, **18**, 193, 1940.
29. GANENE, J. F. *Rev. Med. de Rosario*, **32**, 599, 1942.
30. GULICK, M., SAMUELS, L. T., and DENEL, H. J. *J. Biol. Chem.*, **105**, 29, 1934.
31. ZUNZ, E., and LA BARRE, J. *Arch. Internat. Physiol.*, **48**, 287, 1939.
32. GRIFFITHS, M., MARKS, H. P., and YOUNG, F. C. *Nature*, **147**, 359, 1941.
33. INGLE, D. J. *Endocrinol.*, **29**, 838, 1941.



34. COLLENS, W. B., SLO-BODKIN, S. G., ROSENBLIETT, S., and BOAS, I. C. *J.A.M.A.*, **106**, 678, 1936.
35. LAWRENCE, R. D., and MADDERS, K. *Lancet*, **1**, 601, 1941.
36. INGLE, D. J. *Endocrinol.*, **34**, 361, 1944.
37. GAEBLER, O. H., and TARNOWSKI, S. M. *Endocrinol.*, **33**, 317, 1943.
38. INGLE, D. J., EVANS, J. S., and SHEPPARD, R. *Endocrinol.*, **35**, 370, 1944.
39. DUNN, J. S., SHEEHAN, H. L., and MCLETCHIE, N. E. P. *Lancet*, **1**, 484, 1943.
40. DUNN, J. S., KIRKPATRICK, J., and MCLETCHIE, N. E. P. *J. Path. Bact.*, **55**, 245, 1943.
41. DUNN, J. S., and MCLETCHIE, N. E. P. *Lancet*, **2**, 384, 1943.
42. KINNERSLEY, H. W., and PETERS, R. A. *Biochem. J.*, **23**, 1125, 1929.
43. MACBRIDE, C. M., and ROBERTS, H. K. *J.A.M.A.*, **122**, 1225, 1943.
44. LEVITT, A., and SCHAUS, J. P. *Med. Times*, **70**, 187, 1942.
45. REINER, L., LANG, E. H., IRVINE, J. W., PEACOCK, W., and EVANDS, R. D. *J. Pharmacol. & Exper. Therapy*, **78**, 352, 1943.
46. MACBRIDE, C. M., and REISS, R. S. *J. Clin. Endocrinol.*, **4**, 469, 1944.
47. MALINS, J. M. *Brit. Med. J.*, 4418, 318, 1945.
48. POLATIN, P., and SPOTINIZ, H. *Am. J. Psychiat.*, **99**, 394, 1942.
49. BLUMBERG, A., and BILLIG, O. *Psychiat. Quart.*, **16**, 454, 1942.
50. BAKER, A. B. *Arch. Path.*, **26**, 765, 1938.
51. WORTIS, J. *J.A.M.A.*, **108**, 971, 1937.
52. SEMON, H. C., and HERRMAN, F. *Brit. J. Derm.*, **52**, 123, 1940.
53. ALTSCHUL, A. *J.A.M.A.*, **106**, 1380, 1936.
54. SCHRIK, E. W. *Am. J. Obst. & Gynec.*, **37**, 146, 1939.
55. CRAMER, H. I. *Canad. M.J.*, **47**, 51, 1942.
56. ANDERSON, E., and LONG, J. A. *Endocrinol.*, **40**, 98, 1947.
57. MACBRYDE, C. M. Twenty-Eighth Ann. Meet. of Assn. for Study of Internal Secretions, 1946.
58. SINDONI, A., JR. *Delaware State M.J.*, **18**, 21, 1946.
59. DRAGSTEDT, VAN PROHOSKA and HARMS. *Am. J. Physiol.*, **117**, 175, 1936.
60. DRAGSTEDT. *J.A.M.A.*, **114**, 29, 1940.
61. SPRAGUE, R. G. *Med. Clin. N. America*, **30**, 933, 1946.
62. GODLOWSKI, Z. *Brit. Med. J.*, 4453, 717, 1946.
63. HEY, A. *Ugeskr. Laeg. No.*, **43**, 901, 1945.
64. HALLAS-MØLER, K. "Chemical and Biological Insulin Studies Dissertation", *K. Ugesk. f. laeger*, Copenhagen, 1945.
65. SLESSOR, A., and NICOL, T. *Lancet*, **252**, 820, 1947.

## OESTROGENS

THE term "oestrogen" has been adopted for all substances, regardless of source or chemical composition, which have the capacity of inducing oestrus in the immature female and spayed adult female-animal. The oestrogens at present in clinical use are the naturally-occurring hormones, their derivatives and synthetic oestrogens.

The naturally-occurring oestrogens are steroid compounds chemically related to cholesterol, the bile acids, ergosterol, calciferol and the androgens. Naturally-occurring oestrogens are phenolic. The phenolic hydroxyl group reacts with a diversity of compounds to form esters. The oestrogen esters, when administered subcutaneously or intramuscularly, exert a more prolonged effect than the free hormone, probably because of delayed absorption of the active principle. From exogenously supplied oestrogen, 20 per cent is excreted in the urine and faeces during the first 24 hours after injection. The unexcreted 80 per cent is not stored in the body but destroyed or inactivated, probably by the liver.

## PHYSIOLOGY

The main sites of oestrogen production are probably the ovaries during child-bearing age, and the placenta during pregnancy. Additional amounts of oestrogen are elaborated by the adrenal cortex [1]. It is suggested that oestradiol is the primary oestrogen, and that oestrone and oestriol represent metabolic or excretion products of oestradiol.

Much controversy exists as to which ovarian cells elaborate the oestrogenic hormone. It seems that all ovarian tissue, the theca cells, the interstitial tissue, the theca-lutein cells and the granulosa cells may secrete oestrogen. The most convincing evidence, however, points to the theca cells as the main site of oestrogen production [2, 3, 4, 5].

## OESTROGENIC ACTIVITY IN THE FEMALE

**During Child-bearing Age**

In the normally menstruating woman, follicle maturation with oestrogen secretion takes place under the combined action of FSH and LH (see Chapter I). The action of FSH alone is not sufficient to produce oestrogen secretion [6].

Oestrogen stimulates the functional activity of the basophils of the anterior pituitary gland (increase in mitochondria and golgi

apparatus, and degranulation) which release the FSH [7]. Under the action of this gonadotrophin, further follicular development occurs, with increasing concentration of oestrogen. Increased oestrogen activity, reaching 1,000 I.U. per 24 hours urine [8], stimulates, in addition, the eosinophils of the anterior pituitary which produce the LH, and by this means induce ovulation and luteinization of the granulosa cells with production of progestin; and this in turn stimulates the eosinophils to elaborate additional luteinizing hormone, or perhaps a third gonadotrophic hormone—the luteotrophic principle (see Chapter I). The corpus luteum produces, together with progestin, increasing amounts of oestrogen. About three days before menstruation, a peak in oestrogen and progestin secretion occurs, which presumably ensures basophilic activity with the release of FSH [7]. This is followed by corpus luteum regression and a marked drop in the oestrogen and progestin levels (see Chapter I and Fig. 3).

### **During Pregnancy**

The excretion of oestrogen in the blood and urine increases steadily during the first 8 weeks of gestation. After this point, the excretion rises rapidly to attain its maximum at term [9]. According to some investigators, the rapid increase in excretion starts only after the seventeenth week of pregnancy [10]. During the first 8 months, only a very small proportion of oestrogen is excreted in the “free” form, but a few weeks before the onset of labour the output of “free” oestrogen rises considerably, while that of the combined form diminishes, though this still continues to make up the major part of the total urinary oestrogen [11].

According to Rakoff and others [12], the oestrogens of pregnancy serum are mainly bound to the protein fraction. This is shown by the fact that vigorous and prolonged hydrolysis is required to liberate the combined hormones, that protein-free filtrates are frequently free of any oestrogenic content, and that almost all of the hormones can be recovered from the protein fraction. The oestrogens do not pass through a colloidal membrane and are not set free by tryptic digestion.

### **EFFECTS OF OESTROGENS ON THE FEMALE REPRODUCTIVE ORGANS**

#### **Uterus**

Oestrogen induces growth of the uterus and produces the proliferative changes in the endometrium. It sensitizes the uterus to the



vasoconstrictor principle of the posterior lobe, whereas progestin inhibits the release of the posterior pituitary factors. Oestrogen dilates the endometrial blood-vessels and increases their permeability, preventing undue congestion. Progestin causes a decrease in the uterine muscle tone, enhancing congestion in the endometrium.

Withdrawal of oestrogen and progestin causes cessation of endometrial activity with rapid regression and shrinkage of the mucosa. This results in disproportion between the length of the coiled arteries and the thickness of the endometrium. With further regression of the mucosa, further coils are formed retarding the blood-flow and causing stasis [13]. About 4-24 hours before the onset of bleeding, the coiled arteries become constricted at their bases adjacent to the uterine musculature, with the result that ischemia occurs in the superficial parts of the endometrium, followed by tissue necrosis and bleeding. The constriction of the uterine vessels is probably due to cessation of progestin secretion and its inhibitory effect on the posterior lobe, which allows the release of the vasoconstrictor principle acting on the oestrogen sensitized mucosa.

This concept of the underlying mechanism of menstruation is changed by the recent observation of Kaiser [161] that certain New World monkeys menstruate, although there are no histologically demonstrable arterioles in their endometria. It is suggested that menstruation does not depend on spiral arteries and arterio-venous anastomoses, though their presence modifies and accentuates the process enormously.

According to Smith and Smith [160] premenstrual regression of the endometrium involves formation or liberation of a highly toxic protein which is lethal to laboratory animals when injected in small amounts. The factor is an atypical euglobulin recoverable from menstrual discharge. It is suggested that this is the "bleeding factor" precipitating the menstrual flow. Formation or liberation of the bleeding factor seems to depend wholly on endometrial catabolism caused by hormone deprivation. The authors' investigations indicate that secretion of progesterone depends not on the preceding oestrogen *per se* but on products from the oxidative inactivation of oestrogen.

Progesterone enhances the metabolic conversion of oestradiol to oestrone and on to oestriol, thus decreasing the oxidative inactivation that appears to take place in the reversible oestradiol to oestrone reaction [162]. During the follicular phase of the cycle oestradiol is secreted in increasing amounts as the follicle matures, and there is evidence that some progesterone is also secreted at this time [163] but always in sufficient amounts to push the oestradiol-oestrone-

oestriol reaction predominantly to the right. The result is oxidative inactivation and release of pituitary gonadotrophins, namely, luteinizing hormone (LH) and luteotrophin. These in turn first synergize with follicle-stimulating hormone (FSH) to cause follicle maturation and ovulation, then to cause luteinization, and then to maintain the corpus luteum until sufficient progesterone is being secreted to convert oestradiol and oestrone on to the more stable oestriol [162]. It is this effect of progesterone at the peak of luteal activity which, the authors believe, depresses oxidative inactivation, removes the source of pituitary-luteal stimulation and results in the subsequent regression of the corpus luteum. This regression allows follicular growth and oestradiol secretion to begin again. In the absence of progesterone, during the premenstrual and early menstrual phase, conversion is minimal and oxidative inactivation marked.

In non-ovulatory cycles, bleeding occurs after withdrawal of oestrogen, and in ovulatory cycles after withdrawal of oestrogen and progestin. Menstruation-like bleeding from a proliferative type of endometrium can be induced in spayed monkeys, and in women, by administering a series of oestrogen injections and then abruptly discontinuing the treatment.

It seems that oestrogen causes endometrial breakdown with bleeding only when the oestrogenic level falls below the bleeding threshold. Clinically, uterine bleeding can be produced by the continued administration of small doses reaching the lower bleeding threshold; or by large doses which first prime the uterine mucosa beyond its bleeding phase and are then withdrawn to reach the upper bleeding threshold with consequent endometrial breakdown.

If, however, the daily dose of oestrogen is reduced very gradually, the endometrium regresses without bleeding. Some women may fail to bleed despite a fall in the oestrogen level, but this is almost entirely due to a refractory state of the endometrium which prevents a growth response sufficient to ensure the occurrence of bleeding. Adequate doses of oestrogen usually prevent the breakdown of the oestrogen-primed endometrium. When the mucosa is at the phase of corpus luteum regression it is, though difficult, not impossible to postpone bleeding with larger doses of oestrogen [164].

### **Fallopian Tubes**

Oestrogen increases the height of the tubal epithelium and influences the activity of the tubal musculature. During the follicular phase, the tubes show regular rhythmic contractions which increase at the time of ovulation.



### Vagina

Oestrogen produces increased proliferation and cornification of the epithelial cells with increased glycogen deposition. Glycogen in turn is converted into lactic acid, keeping the vaginal acidity at the approximate pH of 4 to 5.

### Breasts

Oestrogens cause mammary growth at puberty. Experimental evidence suggests that the oestrogens induce duct growth, whereas corpus luteum hormone is required for lobule-alveolar development.

#### OESTROGENIC ACTIVITY IN THE MALE

Oestrogens are produced in the male by the adrenal cortex and presumably by the germinal epithelium of the testes. The excretion rate of oestrogen is between 90–120 I.U. daily in the urine of normal men [14] and 2–17 mouse units per litre in the urine of castrates [15].

Törnblom [16] demonstrated that testosterone, administered in large quantities to castrated rats, did not prevent the pituitary hypertrophy which ordinarily occurs after castration. He concluded that it was not lack of testosterone which produced castrational changes in the pituitary. The application of X-rays to the testicles of rats in doses which produced atrophy of the germinal epithelium but did not affect the interstitial cell tissue, was, he observed, followed by castrational changes in the pituitary—thus demonstrating that the pituitary inhibitory hormone resides in the germinal epithelium. He isolated from testicular tissue the fraction which prevented pituitary weight-increase in the castrate rat and found that it possessed all the biological properties of oestradiol. In this connection it is interesting to note that increased quantities of oestrogen appear in the cock during spring, at a time when testicular activity is at its height [17] (see Chapter IX).

### Spermatogenesis

Min Chueh Chang [18] has studied sperm production and sex drive in two Suffolk rams before and after implantation of diethylstilboestrol. The sperm production increased 7–9 days after implantation, the effects lasting for about 5 days. The number of sperms was proportional to the dosage and the sperms collected were fertile. There was no effect on sex drive or on sperm morphology or quality; the sperms collected showed a high tendency to agglutinate.

The absorption rate of diethylstilboestrol was 4.25–5 mg. daily.



The author suggests that the implantation of this substance stimulates the pituitary to produce gonadotrophic hormones which, in turn, cause the increase of sperm production and accessory secretion.

Two male patients with diminished sexual potency were treated with oestrogenic hormone following a course of testosterone propionate. A third patient received alternate injections of androgenic and oestrogenic hormones. The oestrogenic hormones were thought to increase libido and potency; and in the amounts and proportions used there was no antagonism between the male and female hormones. Spermatogenesis did not occur in patients with total aspermia. The effect of the drug was not maintained over any length of time [19].

#### GENERAL EFFECTS OF OESTROGENS

##### **Calcium Metabolism**

Oestrogens accelerate maturation of the osseous centres and closure of the epiphyses. Increased blood-calcium and bone metaplasia were observed after oestrogen injections [20].

##### **Electrolyte and Water Metabolism**

Oestrogens favour sodium and water retention, causing accumulation of fluid in the extra-cellular spaces, often with the accompaniment of slight oedema of the face and ankles.

##### **Nitrogen Metabolism**

Oestrogens promote nitrogen retention with increased urinary excretion of urea and creatin.

##### **Vascular System**

Oestrogens produce vasodilatation and hyperaemia and an increase of blood volume due to altered electrolyte and water metabolism.

#### INACTIVATION OF OESTROGENS

About 80 per cent of exogenous oestrogen is destroyed or inactivated, presumably by the liver. Heller [21] has suggested that liver tissue contains an oestrone-reducing enzyme system which converts oestrone into oestradiol, and an oestradiol-destroying system which inactivates the oestradiol so formed. He found that oestriol is only mildly affected by the liver, thus explaining why oestriol is more efficacious orally than oestrone or oestradiol. Zondek found that synthetic oestrogens such as diethylstilboestrol are also inactivated by the liver [22]. Aqueous acid liver extracts inactivate both oestrone

and stilboestrol *in vitro*. Alkaline extracts inactivate diethylstilboestrol, but not oestrone [23].

### Vitamin B

The oestrogen inactivating function of the liver is considerably impaired by vitamin B deficiency. In the rat, deficiency of B complex decreases the capacity of the liver to inactivate oestrone and  $\alpha$ -oestradiol [24, 165].

Bean found that cutaneous vascular spiders and palmar erythematata, once mainly associated with cirrhosis of the liver, also occurred in nutritional deficiency and at the period of pregnancy when oestrogens significantly increase. Menorrhagia, metrorrhagia, cystic mastitis, premenstrual tension, and possibly other syndromes related to an excess of oestrogen, were apparently caused by vitamin B deficiency leading to failure of the liver to inactivate oestrogen. Administration of vitamin B complex resulted in prompt improvement.

Vitamin B deficiency causes impairment of liver function only in respect of oestrogens; androgens continue to be inactivated, however, with consequent serious disturbance of the oestrogen-androgen equilibrium [25]. Impaired oestrogen inactivation and vitamin B deficiency form part of a vicious circle, since increase in the amount of oestrogen may itself cause vitamin B deficiency [26].

Recently some doubt has been cast on the rôle of the vitamin B complex in the inactivation of oestrogens. Pellets of oestrone were implanted in the spleen of spayed female rats. While being maintained on a normal diet the animals inactivated the absorbed oestrone, as judged by the anoestrous condition of the vaginal smear. When such animals were placed on a vitamin B-complex-free diet the ensuing acute deficiency decreased the ability of the liver to inactivate oestrone, and the vaginal smears became cornified. Paired inanition control animals, limited to the same amount of food consumed by the deficient animals but receiving B vitamins, also failed to inactivate oestrone. The effect of acute vitamin B-complex-deficiency in producing a failure of the liver to inactivate oestrone seems therefore to be due to the concomitant inanition. In other animals, limited to 3 grams of food a day, but receiving B vitamins, a failure to inactivate oestrone was also encountered. Supplements of methionine were without effect in preventing the occurrence of vaginal cornification in either the animals deficient in B complex or in animals with restricted food intake [166].

Zondek and Finkelstein [167] found that vitamin B-complex-deficient rats are able to inactivate oestrone *in vivo* as are normal

rats; and that whereas the liver of normal rats inactivates oestrone *in vitro* in a high percentage of cases, the liver of vitamin B-complex-deficient rats does not inactivate oestrone *in vitro* (see Chapter XIV).

### Folic Acid

Chicks maintained on a stock diet show a forty-fold hypertrophy of the oviduct following stilboestrol administration, whereas "folic acid"-deficient chicks show only a four-fold increase in oviduct weight following identical treatment. In "folic acid"-supplemented chicks, the degree of oviduct weight-increase varies directly with the level of "folic acid" ingested, but doses of "folic acid" considerably in excess of that required for growth and haemopoiesis fail to restore the oviduct response to the level obtained in chicks on a stock diet. Concentrates containing folic acid from yeast and spinach when given in increasing doses similarly cannot completely replace the stock diet. [168].

Koref and Engel [169] demonstrated that folic acid in relatively large amounts inhibits the oestrogenic activity of oestrone to a certain rather moderate degree. One mg. of folic acid inactivates about 50 I.U. of oestrone.

## THE RELATIONSHIP OF OESTROGEN TO OTHER ENDOCRINE GLANDS (see Figs. 6 and 7)

### Anterior Pituitary Gland

The amount of oestrogen secreted depends to a large extent on the pituitary-ovarian relationship and on the condition of the anterior pituitary gland, although it is also influenced by the functional state of other glands. Both hyperfunction and deficiency of the anterior pituitary cause marked ovarian changes, accompanied by disorders of the menstrual cycle. Functional disturbances in the pituitary which influence ovarian activity may be primary, or due to altered function of other glands. Complete thyroidectomy in the rabbit, for example, causes increased secretion of FSH by the pituitary, followed by follicular hypertrophy [27]. Conversely, ovariectomy or oestrogen deficiency produces heightened secretory activity in the pituitary with the characteristic cytological changes of the basophil cells, frequently accompanied by increased thyroid activity.

The normal fluctuations in the oestrogenic level apparently regulate pituitary function. Whether this effect of oestrogen is one of inhibition or stimulation has for long been a matter of controversy.



On the basis of the available evidence, it appears the oestrogenic effect is not influenced by dosage, and that its duration determines whether it acts on the pituitary by inhibition or stimulation.

Zondek [28] reported enlargement of the pituitary gland due to hypertrophy and hyperplasia of the chromophobes following pro-

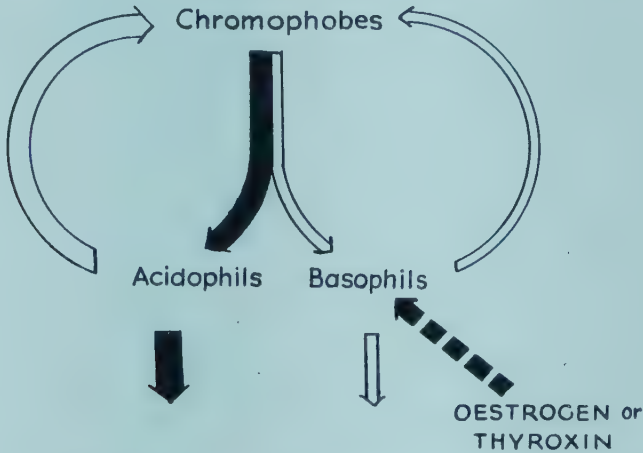


FIG. 6.—THE EFFECT OF INCREASED OESTROGEN OR THYROXIN PRODUCTION ON THE ANTERIOR PITUITARY GLAND

longed treatment with oestrogen. If the treatment is sufficiently prolonged, the basophils become completely degranulated, and the acidophils likewise show loss of granules. When this transformation from a chromophil to a chromophobe is carried to an extreme by

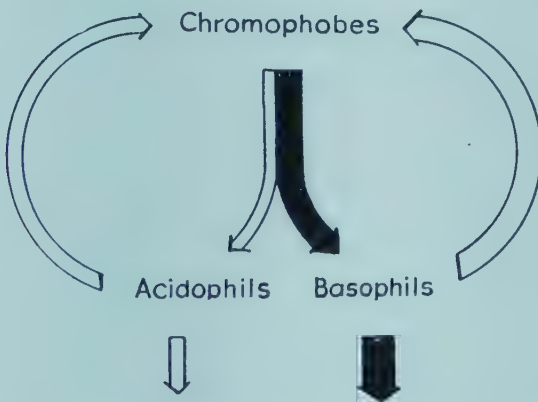


FIG. 7.—THE EFFECT OF OESTROGEN OR THYROXIN-DEFICIENCY ON THE ANTERIOR PITUITARY GLAND

very protracted treatment, large chromophobic adenomata may form and cause clinical manifestations of pituitary insufficiency. Halpern and d'Amour [29] suggested that the depression of the anterior lobe after oestrogen administration may be considered as one aspect of a reaction to intense stimulation. The demands of the stimulus having

exceeded the secretory capacity of the gland, a period of hyperplasia follows. During this period, a process of rapid cellular proliferation occurs, taking up most of the gland's energy, while the secretion of the gonadotrophic hormone is diminished. Clauberg and Breipohl [30] contend that large doses of oestrogen temporarily suppress pituitary activity; but that this, after the withdrawal of the inhibiting factor, returns vigorously with release of the pituitary luteinizing hormone. Engles [31] described the action of oestrogen on the pituitary as a "release phenomenon", suggesting that high doses of oestrogen induce increased hypophyseal activity with release of gonadotrophic hormone. Korenchewsky and associates [32] have shown that the pituitaries of male rats, in which testicular atrophy has been induced with oestrogen, present gross modifications indicative of activation rather than suppression.

Hohlweg [33] and Klaften [34] induced corpus-luteum formation and pituitary enlargement in animals by either a single dose of 500 r.u. of oestrogen, or 60,000–100,000 I.B.U. of oestrogen over a 4-week period. This has since been confirmed by several workers. Chamorro [35] observed that corpora lutea which, in the intact animal, form within 5 days after the institution of oestrogen therapy, fail to appear if the pituitary is removed on the second day of treatment, but do appear if the pituitary is removed on the fourth day. They regard this as evidence that oestrogen stimulates the pituitary to release its luteinizing hormone at some point between the second and fourth day of treatment. Eskin [36] demonstrated that injections of oestradiol benzoate into immature rats produced a release of gonadotrophic hormone from the anterior pituitary gland. Examination of the pituitary glands of the treated animals and the controls revealed that the glands of the former contained far less gonadotrophic substance than those of the latter. The injections of oestradiol benzoate produced corpus luteum formation in every case; but this did not occur when the oestradiol benzoate was injected together with adrenalin.

The pituitaries of adult female rats, under normal oestrogen influence, stimulated moderate luteinization in normal and hypophysectomized recipient immature female rats. The pituitaries of adult female rats freed of oestrogen stimulation through oophorectomy, invariably caused extensive luteinization of the recipient ovaries. This indicated that liberation of the hypophyseal luteinizing factor was minimal in the absence of oestrogen, the factor remaining stored within the pituitary gland.

Release of the luteinizing factor by oestrogen was suggested also

by the type of ovarian response produced by pituitaries from oophorectomized adult female rats which had been injected with oestradiol benzoate daily for 30–45 days. These pituitaries produced follicular development but no corpora lutea, owing to removal of the luteinizing factor by the oestrogen treatment [170].

These results are in harmony with the cytological studies of Severinghaus, who postulated that oestrogen in small doses stimulated increased functional activity of the basophils of the anterior pituitary (increase in mitochondria and Golgi apparatus and degranulation). Larger doses also stimulated the eosinophils in a similar manner, increasing the luteinizing fraction of the anterior pituitary (see Chapter I).

Abarbanel [37] treated a series of patients with excessive functional bleeding from endometria showing cystic glandular hyperplasia, with from 600 to 1,200 mg. of diethylstilboestrol orally over a period of 6–12 weeks. Endometrial biopsies at weekly or bi-weekly intervals disclosed a marked proliferative effect during the course of treatment. Within 5–10 days after discontinuance of oestrogen administration, oestrogen-withdrawal bleeding ensued. Biopsies taken 3–4 weeks later showed, in every case, a strong secretory pattern and, in several instances, a marked decidual reaction. In 10 of 12 such cases, menses became rhythmically cyclic and normal in amount, and follow-up premenstrual endometrial biopsies revealed a secretory pattern.

The effect of oestrogen on the lactogenic hormone is not clear, for reports have been contradictory. Some observers [38, 39] believe that oestrogen does not depress lactation when nursing is normal; others [40, 41, 42, 43, 44] report inhibition of lactation in the same circumstances. Folley and others [45, 46] showed that oestrogen administration to lactating cows exerted a prolonged galactopoietic effect, increasing the concentration of solids in the milk without diminution of yield. Later it was demonstrated that synthetic oestrogen would induce lactation in virgin goats [47], and recent studies in heifers and cows have corroborated earlier claims for the galactopoietic effects of oestrogen [48–50].

The lactogenic activity of oestrogen is explained by its stimulating effect on mammary tissue growth, and its ability to release the lactogenic hormones from the anterior lobe. Folley and co-workers [51] found that administration of anterior pituitary extract in addition to oestrogen initiated lactation more quickly than oestrogen alone. Experiments have shown that small doses of oestrogen increase, whereas large doses inhibit lactation. It is suggested that this effect



is mediated through the pituitary, which is stimulated by small and suppressed by large doses.

Recently, convincing evidence [44] has been given for the inhibitory effect of oestrogen on human lactation. Stilboestrol 1 mg. 3 times daily given to normally nursing mothers depressed milk secretion in spite of normal suckling, but there was no quantitative relation between the dose of stilboestrol and the depression of milk secretion.

On growth hormone, high doses of oestrogen apparently exert an inhibitory action [52].

Since oestrogen stimulates the adrenal cortex of intact animals, but fails to exert this effect in the hypophysectomized animal [53], it is suggested that oestrogen increases the secretion of the adrenocorticotrophic hormone [143, 144].

### **Posterior Pituitary Gland**

Oestrogen appears to sensitize the uterus to the vasoconstrictor principle of the posterior lobe, whereas progestin inhibits its release. After regression of the corpus luteum and the cessation of progestin secretion, the inhibitory effect on the posterior lobe is removed, with consequent release of its vaso-constrictor principle. This acts on the oestrogen-sensitized mucosa, before the onset of menstruation, causing vaso-constriction of the uterine vessels.

### **Thyroid Gland**

During the normal menstrual cycle, the peaks of oestrogen excretion coincide with lower values in the basal metabolic rate [54]. This suggests that, in the normal individual, thyroid activity is somewhat depressed when blood and urine oestrogens are high and vice versa. Clinically, oestrogens have been shown to depress thyroid function and lower the basal metabolic rate [55, 56, 58, 141]. Oestrogens given to rats on low-iodine diet reduced the goitre but caused some degeneration of the thyroid parenchyma and lowered iodine storage [136]. The effect of oestrogen on the gonadal system depends on the state of thyroid function. An excess of thyroid increases the threshold of response to oestrogen. More oestrogen is required to induce oestrus in the thyroid-fed than in the untreated castrate. Likewise, the threshold of response to gonadotrophic and androgenic hormones in animals with hyperthyroidism is increased [59] (see Chapter III).

### Parathyroid Gland

Prolonged administration of oestrogens produces a phenomenon similar to osteitis fibrosa [60]. The increase of blood calcium and of bone metaplasia after oestrogen injections suggests that oestrogen acts through the parathyroid gland [20].

### Pancreas

There is much conflicting evidence as to the influence of oestrogen on the blood-sugar level [62, 63, 64, 65, 66]. Ganene [67], giving oestrone 10,000–50,000 I.U. intramuscularly daily, obtained an improvement in diabetes in 50 per cent of patients. Gerber obtained, by the administration of oestrogens, a reduction of 31.6 per cent in the total daily insulin requirements of diabetic patients with apparently active hyperpituitarism [134]. Others have reported an increase of blood-sugar and liver glycogen [68, 69, 70, 71], or no effect at all [72, 73].

These contradictory results are difficult to explain. It is possible that the corticomimetic effect of oestrogen plays some causal part in the differences, which may further be influenced by the dosage of oestrogen and the length of treatment. Ingle [74], however, demonstrated that the diabetogenic activity of diethylstilboestrol is not mediated through the anterior pituitary or through the adrenal and pituitary glands together. Administration of diethylstilboestrol caused hyperglycaemia and glycosuria in adrenalectomized, hypophysectomized, partially-depancreatized male rats, kept on a sub-diabetogenic regime of adrenal cortical and anterior pituitary extracts, but all the test animals were, he found, nondiabetic during the control periods.

### Adrenal Cortex

Oestrogen is partly produced by the adrenal cortex [1]. Excessive amounts of oestrogen were noted in some cases of virilism and Cushing's Syndrome, which accounts for the feminism associated with adrenal cortical disorders in the male [76, 77].

Injections of oestrogen in moderate doses cause marked hypertrophy (not in the hypophysectomized rat [53]), while excessive doses produce cortical atrophy [78]. It is interesting to note that the changes in the adrenal cortex during the menstrual cycle of normal women, suggesting increased cortical function, coincide with the peaks of oestrogen secretion (see Chapter V).

### Adrenal Medulla

Adrenalin inhibits the stimulating effect of oestrogen on the anterior pituitary gland in the rabbit [36].

### Ovaries

Oestrogen controls ovarian activity through its action on the gonadotrophin-producing cells of the anterior pituitary. Moderate doses of oestrogen, stimulating first the basophils and then the oesinophilic cells, exert a luteinizing effect and inhibit further development of follicles. Prolonged oestrogenic activity, particularly when at high levels, depresses pituitary and ovarian function. It has recently been shown that the stimulating effect of moderate doses on the ovaries is not entirely mediated through the pituitary. Williams maintained normal ovarian weight in the hypophysectomized rat with daily injections of 100  $\mu$ g. of stilboestrol, whereas the ovaries of the control animals were significantly lighter than normal [80].

The metabolism and utilization of progestogen are greatly augmented by oestrogen, when both hormones are given simultaneously in the presence of an oestrogen-primed uterus. Progestogen partially protects oestrogen against inactivation [81] and promotes its excretion [82] (see Chapter VIII).

### Testes

Oestrogens and androgens are present in both sexes, the values of androgen in the female of child-bearing age approaching those in the male [9, 84]. The action of the hormones is apparently synergic as long as they are present in the ratio necessary to maintain the balance of pituitary activity. A significant alteration in the oestrogen/androgen ratio produces an antagonistic relationship of the hormones in both sexes. A shift to increased activity of the opposite sex hormone causes defeminization, with heterosexual alterations in the female and demasculinization in the male. It appears reasonable to assume that a shift in the ratio towards an absolute or relative increase in the isosexual hormone may occur. Careful investigations of such conditions may reveal a number of new aetiological factors in disorders of the pituitary gonadal relationship, and supply a rationale for the therapeutic application of heterosexual hormone preparations (see Chapter IX).



### Vitamin E

Vitamin E is believed to be antagonistic to oestrogen. During pregnancy, oestrogen and vitamin E exist in a sort of equilibrium, and an excess of oestrogen may be taken as evidence of vitamin E deficiency. Vitamin E should therefore not be given on any account in conditions of oestrogen deficiency, since this would further suppress the oestrogenic level [85, 86].

### STANDARDIZATION

Oestrogens were first standardized in terms of rat and mouse units; later, to ensure uniformity, an international unit was established in 1932, and defined as the specific oestrus-producing activity contained in 0.1 gamma of the international standard ketohydroxyestratriene (oestrone) preserved at the National Institute for Medical Research in London. The relation of the biological to the international unit is difficult to define, since different values are reported by various workers. Examples are given in the following table [87].

<i>Author</i>	<i>1 Mouse Unit</i>	<i>1 Rat Unit</i>
Hain and Robson . . . . .	0.9 I.U.	33 I.U.
Schoeller, Dohm and Hohlweg . . . . .	5.0 I.U.	25 I.U.
Pedersen-Bjergaard . . . . .	3.5 I.U.	28 I.U.
Emmens . . . . .	1.5 I.U.	...
Doisy . . . . .	0.5 I.U.	...
d'Amour and Gustavson . . . . .	. . . . .	13 I.U.
Hinglais and Hinglais . . . . .	. . . . .	25 I.U.
Burn . . . . .	. . . . .	9.6 I.U.
Mazer and Israel . . . . .	. . . . .	10.6 I.U.
Gerard . . . . .	. . . . .	41 I.U.
Rowe and Simond . . . . .	. . . . .	3 I.U.

Following the introduction of esters of oestrone and oestradiol for clinical use, differences were noted in rates of absorption, and an international unit for oestradiol benzoate, the so-called International Benzoate Unit (I.B.U.), was established by the Second Conference for the Standardization of Sex Hormones in 1935. This is defined as the specific oestrus-producing activity contained in 0.1 gamma of the International Standard.

### ADMINISTRATION

The oestrogens synthetically prepared, or from natural sources, may be administered in many ways, and great care is called for in the choice of the oestrogens and their method of administration.

### **1. Oral Route**

The oral route is effective, but larger doses have to be given than by injection. The oral use of oestrogens, especially if stilboestrol is used, may produce certain disagreeable symptoms, mainly nausea and vomiting, but sometimes also headache and vertigo. The incidence of these symptoms, however, is small—according to some authors as low as 5 per cent—and it seems to be directly proportionate to the dosage.

### **2. Sub-lingual Route**

Oestradiol in propylene glycol solution. An efficient method of administration is to place a few drops under the tongue on each side of the frenulum, from which it is rapidly absorbed. This application has sometimes been effective within 3–4 days in such surprisingly small doses as 0.2–0.3 mg. daily—and once the clinical symptoms have been controlled, patients have been maintained on as little as 0.1 mg. daily with no untoward effects [88].

### **3. Intramuscular Route**

Injections of oestrogen, mostly in oil, are given by the intramuscular route.

### **4. Absorption by Cutaneous or Mucosal Route**

Oestrogenic solutions, made up in suppositories or ointment, offer great scope for treatment where localized action is required. They are easily absorbed through the skin and mucosal surfaces. They produce not only a systemic, but also an intensified local effect when applied in this manner.

### **5. Implantations**

This method was first used in the human subject by Bishop [89]. It involves subcutaneous implantation of compressed or fused pellets of the crystalline hormone. Oestrone, oestradiol, oestradiol benzoate, oestradiol dipropionate and stilboestrol have been used in cases where prolonged action has been required. According to Bishop and Folley [90] a 100-mg. compressed pellet of oestradiol remains effective for more than 2 years. Generally, however doses from 15 to 50 mg. suffice.

The disadvantage of this method is that the compressed pellet sometimes becomes surrounded by a protective coat of fibrous tissue, and thus ceases to function before complete absorption has taken



place. This apparently is not the case when aqueous suspensions or crystals of the pure hormones are injected [61, 75, 88, 91]. After intramuscular injection of the crystals, the water disperses rapidly and the crystals act in the same manner as the pellets.

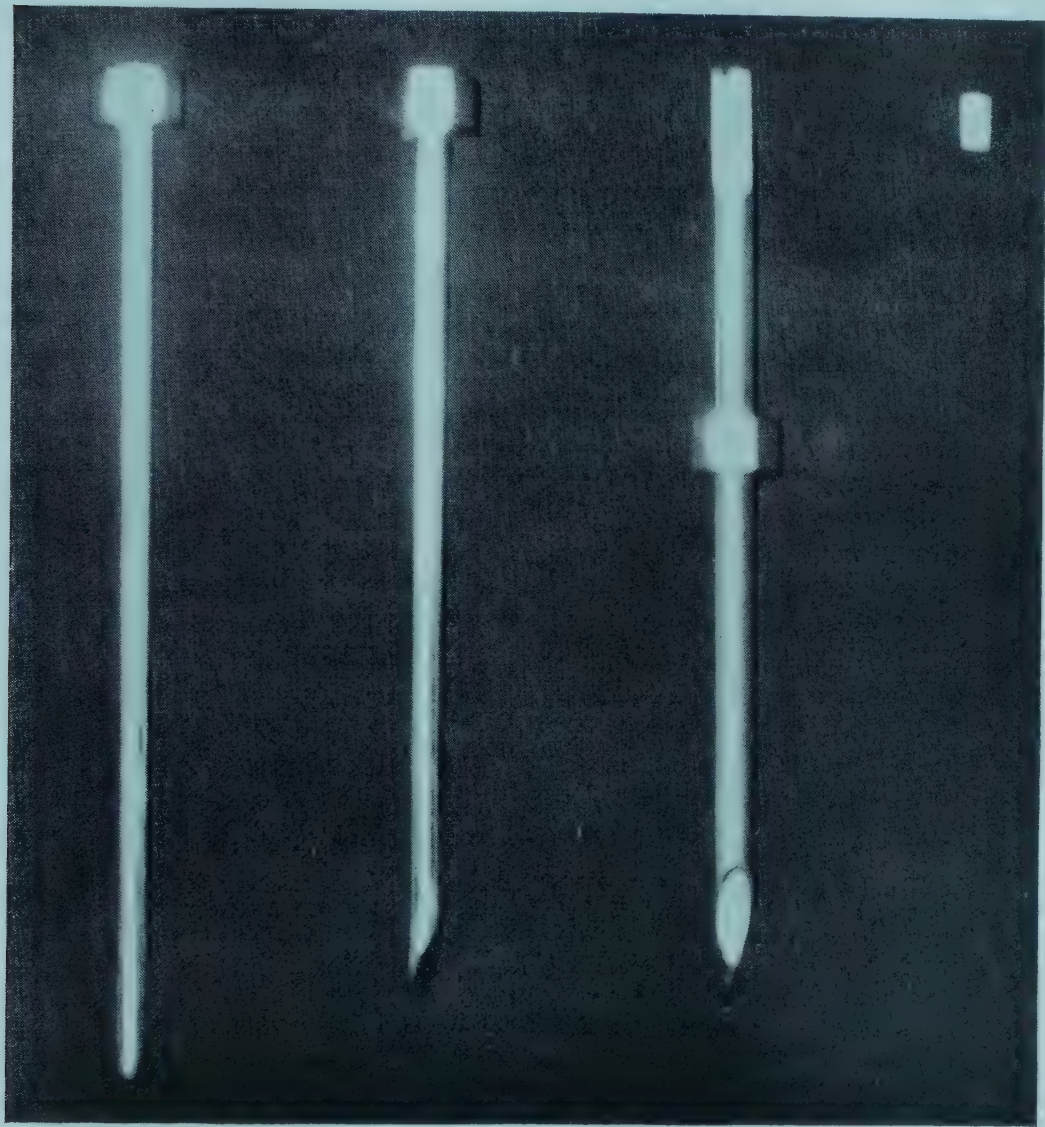


FIG. 8.—KEARN'S PELLET INJECTOR

### *Method of Implantation*

Implantations of fused or compressed pellets are made through an incision of approximately 3 cm. in length between the pubis and the umbilicus. After exposure of the rectus muscle, the pellet is inserted under the sheath to lie on top of the muscle belly. Incisions have also been made just over Poupart's ligament [92].

Linde and Bennett [93] describe their technique of implantation through a 12-gauge hollow needle fitted with a stylet. Up to 7 compressed crystalline oestrone pellets, each 1.83 mm. in diameter and



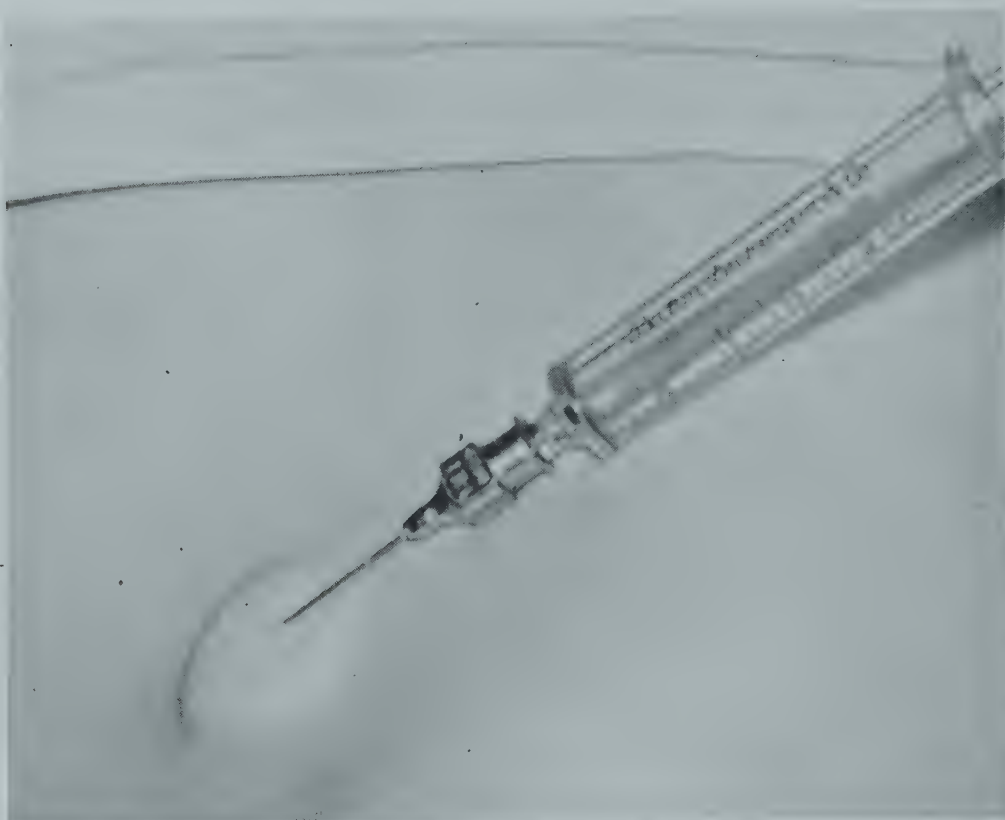


FIG. 9.—INFILTRATION OF PROCAINE



FIG. 10.—INSERTION OF CANNULA AND TROCAR

(Figs. 8-11 by kind permission of Dr. R. B. Greenblatt, Professor of Endocrinology,  
University of Georgia)

varying from 2 to 5 mm. in length and from 5 to 10 mg. in weight, are inserted, by means of a sterile forceps, into the pointed end of the needle after withdrawal of the stylet. After skin sterilization and local anaesthesia, the needle is inserted into the gluteal region obliquely beneath the skin. As the needle is withdrawn, the stylet is pressed in and the pellets are implanted subcutaneously.

The simplest effective method for the implantation of pellets is



FIG. 11.—INSERTION OF PELLETS AFTER WITHDRAWAL OF TROCAR

the following. The skin in the lower quadrant near the inguinal region is cleaned and infiltrated with procaine. A large hollow needle especially devised for implantations and provided with a sharp stylet for penetration and a blunt plunger is pierced through the skin and guided 4–5 cm. towards the inguinal canal. The stylet is then withdrawn. By means of sterile forceps the pellets are placed in the neck of the needle and pushed to the needle point with the blunt plunger.

The needle is withdrawn from about the pellets, which are left in the tract thus created, by removing it until it meets the plunger (stationary), rather than by pushing the pellets out of the end of the needle. As many as six to twelve pellets may be placed in one tract. The small wound is compressed for a moment, painted with an antiseptic and covered with either collodion or flamed adhesive (see Figs. 8-11)

The rate of absorption of any given amount of crystalline hormone depends on the number of pellets implanted at one time and not on the size or weight of the pellets; and the time that the pellets continue to give off appreciable material depends on the size and weight of the pellets implanted [129].

Oestrone crystals in aqueous suspension are injected intramuscularly.

#### OESTROGENS FOR ORAL ADMINISTRATION

##### Oestradiol

(Dihydrotheelin, dihydroxyoestrin,  $\Delta$ -1,3,5:10-oestratriene-3:17- $\alpha$ -diol)  
 $C_{18}H_{24}O_2$

Alpha-oestradiol is the most potent of the naturally-occurring oestrogens by injection. Given orally it has an oestrogenic effect comparable to that of oestrone (ascertained by Mack [94] who studied the oestrogenic activity of various substances by the iodine vapour method of staining vaginal smears for glycogen). Oestradiol has been isolated from sows' ovaries, human placenta, horse testes and the urine of pregnant women and mares.

Range of single dose: 0.1-0.5 mg.

##### Ethinyl Oestradiol

(17-ethinyl-oestradiol)

This is a relatively new preparation about 15-30 times as active as alpha-oestradiol [99], and from 5 to 20 times as active as diethylstilboestrol [95].

Range of single dose: 0.05-0.15 mg.

##### Oestrone

(Theelin, ketohydroxyoestrin, alpha-1,3,5:10-oestratriene-3- $\alpha$ -17-one)  
 $C_{18}H_{22}O_2$

Oestrone, the next most potent naturally-occurring oestrogen, is isolated from stallion testes. It has also been obtained from human pregnancy urine, urine of pregnant mares, stallions and men, from human placenta and adrenal cortex, and from sows' ovaries.



Oral administration generally seems to evoke the more prompt, parenteral administration the more prolonged, reaction, but daily divided doses given by mouth exert a more prolonged effect than the identical total amounts given in an injection [96].

Range of single dose: 1,000–10,000 I.U. equivalent: 0.1–1.0 mg.

### Sodium Oestrone Sulphate

This substance is prepared by the sulphonation of the conjugated oestrogens found in pregnant mares' urine. It appears to have the same oestrogenic action as stilboestrol and has proved to be better tolerated [95, 96, 97].

Range of single dose: 1.0–3.75 mg.

### Oestriol

(Theelol, trihydroxyoestrin,  $\Delta^{1,3,5:10}$ -oestratriene-3,16,17-triol)  $C_{18}H_{24}O_3$   
Oestriol is orally the most potent oestrogen. It occurs in conjugated form with glycuronic acid in pregnancy urine and in human placenta. Mack, however, on the basis of the vaginal glycogen index, found that in the human oestriol is less effective than an equal weight of oestrone [94].

Range of single dose: 200–400 I.U.

### Stilboestrol

(Trans-4:4' Dihydroxy- $\alpha\beta$ -diethylstilbene)  $C_{18}H_{22}O_2$

Stilboestrol, synthesized by Dodds and associates, is highly oestrogenic. It is orally about as effective as oestradiol given subcutaneously. The Council of Pharmacy and Chemistry has adopted the name "Stilboestrol" for the mother substances of 4:4'-dihydroxystilbene, and the name "Diethylstilboestrol" for its more potent derivative 4:4'-Dihydroxy-Alpha: Beta-Diethylstilbene. These substances are not steroids, but have, like the naturally-occurring oestrogens, phenolic properties.

This is the most widely used synthetic oestrogen for oral purposes. It produces a glycogen response more quickly, but also more fluctuating, than do equal weights of oestrone or oestradiol [94]. Untoward effects such as nausea, vertigo and vomiting have been reported from its administration. It appears, however, that these are mainly due to excessive doses, the amount employed determining the grade of toxicity. A recent extensive series of observations afforded no evidence of harmful changes from massive diethylstilboestrol therapy, by doses ranging from 1.0 to 5.0 mg. orally daily, increased in some cases to 100.0 mg. daily in non-pregnant women. Diethyl-

stilboestrol produced no nausea in pregnant women until a dose of 500.0 mg. per day was reached [101].

Range of single dose: 0.1–5.0 mg.

### Hexoestrol

(Meso 4:4' dihydroxy- $\gamma\delta$ -diphenyl-n'-hexane)

This oestrogenic substance, synthesized by Campbell, Dodds and Lawson [102] shortly after the discovery of stilboestrol, has about one-fifth to one-tenth the effectiveness of stilboestrol [103]. It is less toxic than stilboestrol.

Range of single dose: 0.5–25 mg.

### Dienoestrol

(3:4 pp'-dihydroxy-diphenyl-2:4-hexadiene)

This substance, synthesized by Dodds and others [104] is about 3–10 times as active as stilboestrol and far less toxic.

Range of single dose: 0.05–0.1 mg. [105].

### Benzoestrol (Octofollin)

(2,4-di(p-hydroxyphenyl)-3-ethyl hexane)

This synthetic oestrogen, isolated in 1942, is comparable with hexoestrol in potency and appears to be non-toxic. Its use has been reported by Talisman, Roberts, Murphy and others [98, 99, 106–8].

Range of single dose: 1.0–5.0 mg.

### D.B.E.

( $\alpha\alpha$ -di-(p-ethoxyphenyl)- $\beta$ -phenyl-bromo-ethylene)

This is a synthetic oestrogen with a prolonged action. It was first synthesized by Robson and Schoenberg [57] and has recently been clinically investigated by Greene [110] in the treatment of menopausal symptoms and carcinoma of the prostate. A loading dose of 1 or 2 grams secured relief from menopausal symptoms for two or three weeks which was maintained by a weekly dose of 100–300 mg., in 6 out of 9 patients. No beneficial effects were obtained in carcinoma of the prostate, though stilboestrol produced marked improvement. With a similar dosage Way [79] obtained beneficial results in some but not all menopausal patients. Those who failed to respond to D.B.E. were completely relieved within three weeks by stilboestrol therapy.

Range of initial dose: 1–2 g.

Range of maintenance dose: 100–300 mg.

## OESTROGENS FOR SUBLINGUAL ADMINISTRATION

**Oestradiol in Propylene Glycol Solution**

The advantages of sublingual application are that the oestradiol is better absorbed than by the gastro-intestinal tract, and only very small doses are required. It is reported that 0.2–0.3 mg. daily, and a maintenance dose of 1.0 mg. daily, are sufficient [88].

## OESTROGENS FOR INTRAMUSCULAR ADMINISTRATION

**Oestrone**

This oestrogen may be employed when moderate dosage, rapid absorption, and quick action are called for.

Range of single dosage: 0.2–1.0 mg. (equivalent to 2,000–10,000 I.U.)

**Alpha-oestradiol Benzoate**

This oestrogen is more oil-soluble and more slowly absorbed than oestradiol itself, and therefore therapeutically more efficient. 1.0 mg. contains 10,000 I.B.U. of oestradiol benzoate.

Range of single dose: 1.0–10 mg.

**Alpha-oestradiol Dipropionate**

This substance has a still more prolonged effect than oestradiol benzoate. Relatively large doses are given.

Range of single dose: 1.0–10 mg.

**Diethylstilboestrol Dipropionate**

As it is oil-soluble this can be used for intramuscular injection. Its action is similar to that of stilboestrol, but is less intense and more prolonged.

Range of single dose: 0.1–5.0 mg.

## OESTROGENIC SUPPOSITORIES

The suppositories usually contain from 1,000 to 10,000 I.U. of oestrone. 1,000 I.U. are equivalent to 0.1 mg.

Range of single dose: 1,000–10,000 I.U.

**Alpha-oestradiol**

One pessary usually contains 1,000 I.B.U. of alpha-oestradiol benzoate.

Range of single dose: 1,000 I.B.U.



### Diethylstilboestrol

Doses as small as 0.1 mg. have proved effective in combating menopausal syndromes. Twenty-six per cent of those treated with diethylstilboestrol suppositories experienced such side effects as slight vaginal soreness and mild nausea: these were never severe [112].

Range of single dose: 0.02–0.5 mg.

### OESTROGENIC OINTMENTS

#### Oestrone

One gram of ointment contains 0.5 mg. (5,000 I.U.) of oestrone (1 mg. is equivalent to 10,000 I.U. of oestrone).

Range of single dose: from 5,000 I.U.

#### Alpha-oestradiol

One gram of ointment contains 0.1 mg. of alpha-oestradiol.

#### Oestradiol Benzoate

One gram of ointment contains 2 mg. or 20,000 I.B.U. of oestradiol benzoate.

#### Diethylstilboestrol

Diethylstilboestrol dissolved in 80 per cent of alcohol has proved effective in percutaneous application.

Range of single dose: 1 mg.–1 c.c.

### OESTROGENS FOR IMPLANTATION

#### Oestrone

Implantation of a 50-mg. pellet is effective for about 4 months [93].

Range of single dose: 15–50 mg. pellet.

#### Oestrone

(Crystals in aqueous suspension)

As the compressed pellets sometimes become surrounded by a protective coat of fibrous tissue and cease to function before complete absorption, Salmon and Geist [88, 91] injected an aqueous suspension of oestrone crystals intramuscularly and found that after the injection the water dispersed rapidly and the crystals acted in the same manner as pellets.

### Oestradiol

A 100-mg. compressed pellet remains effective for more than 2 years [90].

### Oestradiol Benzoate: Oestradiol Dipropionate

Pellets from 4 to 50 mg. [91]. The average absorption rate of a 15-mg. pellet of oestradiol benzoate is about 0.05 mg. per day [146].

#### CLINICAL APPLICATION AND APPROXIMATE DOSAGES

Oestrogenic therapy may be employed for either its substitutional or its pharmacological effect. In addition it appears that oestrogens in small or large doses over a short period stimulate, and in moderate and large doses over a protracted period inhibit, pituitary function (see p. 140). The direct effect of oestrogens is probably exerted on the basophil cells, and stimulation or inhibition of the eosinophils is determined by the state of basophil cell activity. Ovarian function is not stimulated but rather inhibited by oestrogens. These oestrogenic effects and their action on other endocrine glands have to be considered whenever oestrogenic therapy is employed.

### Delayed Puberty

Oestradiol Dipropionate: 10 mg. twice weekly, followed, after a period of successfully established menses, by twice-weekly injections during the first fortnight of the cycle only.

or: Hexoestrol: 5 mg. 3 times daily, followed, after a period of successfully established menses, by similar treatment during the first fortnight only.

### Sexual Infantilism

Stilboestrol: 0.5–1.0 mg. daily until the uterus reaches normal size. On attainment of nearly normal size, cyclic therapy is indicated [119].

### Amenorrhoea (Hypo-oestrogenic)

Diethylstilboestrol: 3 mg. daily

or: Oestrone Sulphate: 3.75 mg. daily.

With both substances the procedure is the same. The total dose is given daily by mouth for 20 days [121] and followed by 10 treatment-free days. Subsequently, similar 30-day cycles are repeated as long as results are desired.

Others [122, 147] advocate, following oestrogen during the first 20 days, the administration of progestogen during the last 10 days of repeated 30-day cycles. Progestogen may be given sublingually in the form of Ethisterone 30–60 mg. daily.

Release of the FSH and LH fractions from the pituitary is controlled by the ovarian hormones, and normal cyclic functioning of the pituitary may accordingly be obtained by cyclic oestrogen-progestogen treatment.

The following are simplified procedures for the induction of bleeding, but their therapeutic value is questionable.

Oestrone: 5.0 mg.  
and Ethisterone: 60.0 mg. } orally daily for 5 days.

Zondek [123].

or: Oestradiol Benzoate: 2.5 mg.  
and Progesterone: 12.5 mg. } intramuscularly for 2 days.

Finkler and Newark [124].

Oestrogens are contraindicated in hyperoestrogenic (hyperfollicular or polyfollicular) amenorrhoea.

### **Hypomenorrhoea and Oligomenorrhoea**

Oestradiol Dipropionate: 1–5 mg. intramuscularly 2 or 3 times a week for 21 days.

or: Stilboestrol: 1 mg. orally daily for 21 days.

Cyclic bleeding may be induced by repeating the treatment after each induced flow [9].

### **Menorrhagia, Metrorrhagia, Metropathia Haemorrhagica**

#### **1. Haemostasis**

Diethylstilboestrol: 5 mg. daily by mouth stops bleeding in 2–7 days [122, 127].

Hexoestrol: 100–250 mg.

Ethinyl oestradiol 0.3 mg. orally daily stops bleeding within 6 days after onset of treatment.

Bickers [145].

or: Diethylstilboestrol: 10–50 mg.

Injected intramuscularly stops bleeding from 2 to 8 hours.

Injections into the cervix are said to be more effective.

Karnaky [126].

Oestrogens serve to secure haemostasis not only in cases of bleeding due to low oestrogenic activity, but also in uterine bleeding caused



by increased or protracted oestrogenic action. Bleeding in the latter case is apparently also due to a fall of the oestrogenic level below the bleeding threshold. It seems probable that protracted oestrogenic action, following the inhibition of FSH production, finally exhausts the LH-producing cells, with a consequent fall in the oestrogenic level and spontaneous bleeding. Cessation of bleeding is obtained by raising the oestrogenic level above the bleeding threshold.

## 2. *Treatment*

Ethinyl Oestradiol: 0.3 mg. for 20 days [156].

Diethylstilboestrol: 3 mg. daily for 20 days  
followed by:

Ethisterone: 60 mg. sublabially every day for 10 days.

In the case of metrorrhagia and metropathia haemorrhagica, 30-day cycles are repeated until endometrial biopsy reveals secretory changes. If the test after 5 cycles is negative, 1-2 cyclic gonadotrophin treatment is advocated [100, 121, 122].

### **Ovarian Cyst** (up to 5 cm. in diameter)

Diethylstilboestrol: 5.0 mg. orally every night generally results in regression of the cyst to a size that cannot be felt by pelvic examination. If no reduction in size occurs after 20 days' treatment, laparotomy is indicated [131] (see p. 146).

### **Dysmenorrhoea**

Hexoestrol: 3-6 mg. 2 or 3 times daily [132]

or: Stilboestrol: 2-3 mg. orally for 21-24 days of the cycle [133].

It is claimed that the beneficial effect of oestrogens results from inhibition of ovulation.

### **Frigidity** (in oestrogen deficient cases)

Oestradiol Dipropionate: 1-5 mg. }  
and: Testosterone Propionate: 10-25 mg. } 2 or 3 times weekly [135].

### **Non-patent Fallopian Tubes**

Oestradiol Dipropionate: 5 mg. (50,000 I. U.) in 5 injections at 5-day intervals [109].

### Induction of Labour

Oestrone: 50,000–250,000 I.U.

or: Oestradiol Benzoate: 2 mg. parenterally at hourly intervals for 10 doses [113].

This is followed by injections of pitocin the day after the cessation of oestrogen administration at 20- to 30-minute intervals for several hours. The initial dose should not exceed 1 or 2 minims (0.6–0.12 c.c.).

Reddoch and Wiener [114] claim 41 per cent successful results with:

Stilboestrol: 600–720 mg. (total dosage),  
followed by small doses of pitocin 3 hours after the oil and enema.

### Lactation (Inhibition)

Stilboestrol: 5 mg. every 4 hours for 3–5 days in the non-nursing mother [116].

### Breast Engorgement

Stilboestrol: 10, 25 or even 50 mg. initial dose, depending upon whether oestrogen is being given to prevent or relieve breast engorgement. The initial dose is rapidly reduced to 5 or even 1 mg. over a period of 7–10 days [117, 142].

### Menopausal Symptoms

Diethylstilboestrol: 0.5 mg. orally daily.

Heller and co-workers [137].

These authors suggest that the dose of 0.5 mg. daily stimulated pituitary function, possibly due to increases in the luteinizing fraction. Doses of 5 mg. apparently suppressed pituitary function, but such large doses were rarely required, the smaller dose of 0.5 mg. generally sufficing to alleviate menopausal symptoms.

Hexoestrol: 0.5–1 mg. in aqueous suspension once a week.  
and in severe cases

1.5–2 mg. in aqueous suspension once a week.

Ersner and co-workers [138].

Oestrone sulphate: 1.2 mg. daily in three divided doses followed by 1.25 mg. daily 3–5 times weekly for maintenance.

Freed [139] and others [129, 130].

Octoföllin: 1–20 mg. orally daily.

Murphy [106].

or: 2 mg. parenterally twice a week for 1 month, then 5 mg. a week and later every 2 weeks for 2 months more completely relieves hot flushes, sweats and dizziness. This treatment also brings about great reduction in menopausal hypertension.

Hufford [107].

Dienoestrol: 0.1 mg. twice daily.

Bishop and Barnes [83, 105]. Others [111, 115] have advocated an initial dose of 0.2-0.5 mg. daily followed by 0.3 mg. daily for maintenance.

Ethinyl oestradiol: 0.05 mg. orally daily.

Wiesbader and others [156].

D.B.E.: 1-2 grams initial dose followed by:

100-300 mg. orally once a week [79].

Greene [110] and Way [79].

### **Involutional Melancholia**

Many workers [148, 149, 150, 94, 21] record beneficial results with oestrogen treatment.

Diethylstilboestrol: 4 mg. daily reduced by 1 mg. weekly to: 2 mg. daily for the remainder of the treatment.

or: Oestradiol benzoate: 1.66 mg. 4 times weekly, reducing the dose to twice weekly.

Heller [21].

### **Senile or Atrophic Gingivitis**

Diethylstilboestrol: ointment massaged into the gum tissues with rubber applicators.

or: Oestradiol dipropionate: injected directly into the muco-buccal fold adjacent to the area to be treated.

Richman [151].

### **Senile Vaginitis**

Diethylstilboestrol: 10 mg. in ointment applied locally for 1 week.

Buxton [152].

Karnaky [132].



### Pruritus Vulvae

Hexoestrol: 3–6 mg. 2–3 times daily.

Oestrone: 2,000–5,000 I.U. in ointment applied twice daily as vulval inunction.

and: Oestradiol benzoate: 30,000 I.B.U. on alternate days with gradual decrease of dosage.

Course of treatment: 8–12 weeks.

Klaften [153].

### Kraurosis Vulvae

Diethylstilboestrol: 10 mg. in ointment applied locally for 1 week.

Buxton [152].

### Acromegaly (*see p. 5*)

Oestradiol benzoate: 1.5–10 mg. intramuscularly daily; followed by:

Oestradiol benzoate: 1 mg. intramuscularly 5 times weekly

or: Oestrone: 1–1.5 mg. orally daily.

Hutton and Reiss [155].

### Hyperthyroidism

The oestrogenic hormone appears to have some inhibitory effect on the thyroid. Farbman [58] advocates oestrogenic therapy for the following conditions:—

1. Post-operative residual hyperthyroidism.
2. Borderline or mild thyrotoxicosis with little or no enlargement of the thyroid gland.
3. Hyperthyroidism arising during the menopause.
4. Pre-operative and post-operative treatment of surgical cases.

### Carcinoma of the Breast

Diethylstilboestrol: 1–2 mg. orally daily [157];

or 6–10 mg. orally daily [111].

Ethinyl oestradiol: 0.15–0.7 mg. orally daily.

It appears that favourable results are obtained predominantly in women above the age of 60, while oestrogens seem to have a deleterious effect in women in the younger age group.

Herrmann, Adair and Woodard [158].

## OESTROGENIC THERAPY IN MAN

**Carcinoma of the Prostate**

Stilboestrol: 3-10 mg. daily for 3 weeks after which it is decreased to 1-3 mg. daily.

Fluhmann [116].

or: 5 mg. (1 c.c.) intramuscularly twice or three times a week.  
and: not less than 10 mg. a day intramuscularly in cases of excessive growth with numerous metastases.

Walker [154]. Others [120, 121, 127, 140, 159].

or: Ethinyl oestradiol: 0.25 mg. to 0.5 mg. daily [130].

**Benign Hypertrophy of the Prostate**

Diethylstilboestrol: 1 mg. 3 times daily up to 10 mg. daily.

Heckel [125].

In carcinoma of the prostate, the serum acid-phosphatase is usually high. Oestrogens produce a lowering of the serum acid-phosphatase with a coincident improvement in the clinical condition.

**Chronic Haemospermia**

Ethinyl oestradiol: 0.05 mg. from 3 times a week to twice daily, depending on the response obtained, for a period of 3-7 weeks.

Huggins and McDonald [128].

## BIBLIOGRAPHY

1. BEALL, D. *Nature*, **144**, 76, 1939; *J. Endocrinol.*, **281**, 1940.
2. ZONDEK, B., and ASCHHEIM, S. *Klin. Wchnschr.*, **6**, 248, 1927.
3. ZONDEK, B. "Die Hormone des Ovariums und des Hypophysenvorderlappens", J. Springer, Berlin, 1931.
4. SELYE, H., COLLIP, J. B., and THOMSON, D. Z. *Proc. Soc. Exper. Biol. & Med.*, **30**, 780, 1933.
5. GREEP, R. O., VAN DYKE, H. B., and CHOW, B. F. *Endocrinol.*, **30**, 627, 1928.
6. FEVOLD, H. L. *Endocrinol.*, **28**, 33, 1941.
7. SEVERINGHAUS, A. E. *Anat. Rec. 61 (Suppl.)*, **61**, 1935; *Physiol. Rev.* **17**, 566, 1937.
8. D'AMOUR, F. E. *J. Clin. Endocrinol.*, **3**, 41, 1943.
9. HOFFMAN. "Female Endocrinology", W. B. Saunders Co., Philadelphia, 1944.

10. FRANK, R. T., and GOLDBERGER, M. A. *Am. J. Obst. & Gynec.*, **43**, 865, 1942.
11. COHEN, S. L., MARRIANS, J. F., and WATSON, M. *Lancet*, **1**, 674, 1935.
12. RAKOFF, A. E., PASCHKIS, K. E., and CANTAROW, A. *Am. J. Obst. & Gynec.*, **46**, 856, 1943.
13. DARON, G. H. *Am. J. Anat.*, **58**, 349, 1936.
14. KOCH, F. C. *Ann. Int. Med.*, **11**, 297, 1937.
15. ENG, H. *Klin. Wchnschr.*, **15**, 349, 1936.
16. TÖRNBLOM. "Uppsala Lak Foren", 1942, Haft 1, Och. 2, p. 1.
17. FALIN GRONTZEVA, K. E. *Bull. Exper. Biol. & Med. U.S.S.R.* **16**, 7-8, 68, 1943.
18. MIN CHUEH CHANG. *J. Endocrinol.*, **3**, 192, 1942.
19. GOLD, S. *Canad. M.A.J.*, **48**, 241, 1943.
20. BACH, E. *Klin. Wchnschr.*, **16**, 218, 1937.
21. HELLER, C. G. *Endocrinol.*, **26**, 619, 1940.
22. ZONDEK, B., SALMON, F., and SKLOW, J. *Endocrinol.*, **33**, 333, 1943.
23. ENGEL, P., and ROSENBERG, E. *Endocrinol.*, **39**, 44, 1945.
24. SEGALOFF, ALBERG, and SEGALOFF, ANN. *Endocrinol.*, **34**, 346, 1944.
25. BISKIND, MORTON, S., and BISKIND, R. G. *Endocrinol.*, **32**, 97, 1943.
26. ASHWORTH, J., and SUTTON, D. C. *Arch. Int. Med.*, **69**, 15, 1942.
27. CHU, J. P., and LEE, C. C. *Proc. Chinese Physiol. Soc., Chengtu Branch*, **1**, 62, 1942.
28. ZONDEK, B. *Am. J. Obst. & Gynec.*, **33**, 979, 1937.
29. HALPERN, S., and D'AMOUR, F. E. *Am. J. Physiol.*, **115**, 229, 1936.
30. CLAUBERG, C., and BREIPOHL, W. *Klin. Wchnschr.*, **14**, 119, 1935.
31. ENGLES, E. T. *Am. J. Physiol.*, **88**, 101, 1929.
32. KORENCHEVSKY, V., and DENNISON, M. *Biochem. J.*, **28**, 1474, 1934.
33. HOHLWEG, W. *Klin. Wchnschr.*, **13**, 92, 1934.
34. KLAFTEN, E. *Ztschr. f. Geburtsh. u. Gynäk.*, **115**, 64, 1937.
35. CHAMORRO, A. *Klin. Wchnschr.*, **16**, 196, 1937.
36. ESKIN, I. A. *Bull. Exper. Biol. & Med. U.S.S.R.*, 7-8, 68, 1944.
37. ABARBANEL, A. R. Ann. Meet. of the Society for the Study of Internal Secretions, Chicago, 1945.
38. ABARBANEL, A. R., and GOODFRIEND, M. J. *Am. J. Obst. & Gynec.*, **40**, 1037, 1940.
39. KARNAKY, K. J. *Am. J. Obst. & Gynec.*, **41**, 565, 1941.
40. STEWART, H. L., JR., and PRATT, J. P. *Am. J. Obst. & Gynec.*, **41**, 555, 1941.
41. DIDDLE, A. W. *Am. J. Obst. & Gynec.*, **41**, 563, 1941.
42. DAVIS, M. E. *Am. J. Obst. & Gynec.*, **41**, 564, 1941.
43. CONNALLY, H. F., JR. *Am. J. Obst. & Gynec.*, **46**, 125, 1943.
44. FIELDS, H. *Am. J. Obst. & Gynec.*, **49**, 385, 1945.
45. FOLLEY, S. J. *Biochem. J.*, **30**, 2262, 1936.
46. FOLLEY, S. J., SCOTT WATSON, H., and BOTTOMLEY, A. C. *J. Dairy Research*, **12**, 1, 1941; *ibid.*, **12**, 241, 1941.



47. FOLLEY, S. J., and MALPRESS, F. H. *J. Endocrinol.*, **4**, 1, 1944; *ibid.*, **23**, 1, 1944.
48. FOLLEY, S. J., STEWART, D. L., and YOUNG, F. G. *J. Endocrinol.*, **4**, 43, 1944.
49. HAMMOND, J., Jr., and DAY, F. T. *J. Endocrinol.*, **4**, 54, 1944.
50. PARKES, A. S., and GLOVER, R. E. *J. Endocrinol.*, **4**, 90, 1944.
51. FOLLEY, S. J., MALPRESS, F. H., and YOUNG, F. G. *J. Endocrinol.*, **4**, 181, 1945.
52. ZONDEK, B. *Lancet*, **2**, 842, 1936.
53. ELLISON, E. T., and BURCH, G. O. *Endocrinol.*, **20**, 746, 1936.
54. HITCHCOCK, F. A., and WARDELL, F. A. *J. Nutr.*, **2**, 203, 1929.
55. GESSLER, C. *Arch. Internat. de Pharmacodyn et de Therap.*, **54**, 263, 1936.
56. SHERWOOD, T. C., SAVAGE, M., and HALL, J. F. *Am. J. Physiol.*, **105**, 241, 1933.
57. ROBSON, J. M., and SCHÖNBERG, A. *Nature*, **150**, 22, 1942.
58. FARBMAN, A. A. *J. Clin. Endocrinol.*, **4**, 17, 1944.
59. SMELSER, G. K. *Anat. Rec.*, **73**, 273, 1939.
60. SILBERBERG, M. and R. *Arch. Path.*, **28**, 340, 1939; *ibid.*, **31**, 85, 1941.
61. GROPER, M. J., and BISKIND, G. R. *J. Clin. Endocrinol.*, **2**, 703, 1942.
62. HENLEY, U. *New Zealand M.J.*, **39**, 308, 1940.
63. BARNES, B. O., REGAN, J. F., and NELSON, W. O. *J.A.M.A.*, **101**, 926, 1933.
64. KURZROK, R. "The Endocrines in Obstetrics and Gynaecology", Williams & Wilkins, Baltimore, 1938.
65. SPIEGELMAN, A. R. *Proc. Soc. Exper. Biol. & Med.*, **43**, 307, 1940.
66. WILDER, R. M. "Hyper-insulinism, Definition and Diagnosis", *Mississippi Doctor*, **18**, 913, 1940.
67. GANENE, J. F. *Rev. Med. de Rosario*, **32**, 599, 1942.
68. GULICK, M., SAMUELS, L. T., and DENEL, H. J. *J. Biol. Chem.*, **105**, 29, 1934.
69. ZUNZ, E., and LA BARRE, J. *Arch. Internat. Physiol.*, **48**, 287, 1939.
70. GRIFFITHS, M., MARKS, H. P., and YOUNG, F. C. *Nature*, **147**, 359, 1941.
71. INGLE, D. J. *Endocrinol.*, **29**, 838, 1941.
72. COLLENS, W. S., SLO-BODKIN, S. G., ROSENTLIETT, S., and BOAS, L. C. *J.A.M.A.*, **106**, 678, 1936.
73. LAWRENCE, R. D., and MADDERS, K. *Lancet*, **1**, 601, 1941.
74. INGLE, D. J. *Endocrinol.*, **34**, 361, 1944.
75. SOULE, S. D. *Am. J. Obst. & Gynec.*, **45**, 315, 1943.
76. SIMPSON, S. L., and JOLL, C. A. *Endocrinol.*, **22**, 595, 1936.
77. SAPHIR, W., and PARKER, M. L. *J.A.M.A.*, **107**, 1286, 1936.
78. BOURNE, J., and ZUCKERMAN, S. *J. Endocrinol.*, **2**, 283, 1940.
79. WAY, S. *Brit. Med. J.*, **1**, 10, 1946.
80. WILLIAMS, P. C. *J. Endocrinol.*, **4**, 125, 1945.
81. PINCUS, G., and ZAHL, P. A. *J. Gen. Physiol.*, **20**, 879, 1937.
82. SMITH, G. V. S., and SMITH, O. W. *Am. J. Physiol.*, **98**, 578, 1931.

83. BISHOP, P. M. F. *J. Obst. & Gynec. Brit. Emp.*, **51**, 51, 1944.
84. KOCH, F. U. *Biol. Symposia*, Vol. IX, p. 41, Cattell Press, Lancaster, Pa., 1942.
85. SHUTE, EVAN. *Surg. Gynec. Obst.*, **75**, 515, 1942.
86. SHUTE, EVAN. *J. Obst. & Gynec. Brit. Emp.*, **44**, 121, 1937.
87. FREED, S. C. "Glandular Physiology and Therapy", Am. Med. Assn., Chicago, 1942.
88. SALMON, U. J., and GEIST, S. H. *Proc. Soc. Exper. Biol. & Med.*, **41**, 2220, 1941.
89. BISHOP, P. M. F. *Brit. Med. J.*, **1**, 939, 1938.
90. BISHOP, P. M. F., and FOLLEY, S. J. (unpublished). Quoted by BISHOP, P. M. F. *J. Obst. & Gynec. Brit. Emp.*, **51**, 51, 1944.
91. GEIST, S., and SALMON, U. J. *N.Y. State J. Med.*, **41**, 2220, 1941.
92. MICHEL, D. B. *Am. J. Obst. & Gynec.*, **41**, 1009, 1941.
93. LINDE, R. W., and BENNETT, H. G. *J. Clin. Endocrinol.*, **3**, 417, 1943.
94. MACK, H. C. *Am. J. Obst. & Gynec.*, **45**, 402, 1942.
95. TURNER, V. H., DAVIS, C. D., and HAMBLIN, E. C. *J. Clin. Endocrinol.*, **3**, 455, 1943.
96. GRAY, L. A. *J. Clin. Endocrinol.*, **3**, 92, 1943.
97. GLASS, S. Y., and ROSENBLUM, G. *J. Clin. Endocrinol.*, **3**, 95, 1943.
98. TALISMAN, M. R. *Am. J. Obst. & Gynec.*, **46**, 534, 1943.
99. ROBERTS, H. K. *J.A.M.A.*, **123**, 261, 1943.
100. HAMBLIN, E. C., HURST, D. V., and CUYLER, W. K. *Am. J. Obst. & Gynec.*, **45**, 512, 1943.
101. KARNAKY, K. J. *J. Clin. Endocrinol.*, **5**, 279, 1945.
102. CAMPBELL, N. R., DODDS, E. C., and LAWSON, W. *Nature*, **142**, 1021, 1938.
103. FREED, S. C., EISIN, W. N., and GREENHILL, J. P. *J. Clin. Endocrinol.*, **2**, 213, 1942.
104. DODDS, E. C., GOLDBERG, L., LAWSON, W., and ROBINSON, R. *Nature*, **142**, 34, 1938.
105. BARNES, J. *Brit. Med. J.*, **1**, 79, 1944.
106. MURPHY, J. A. *Am. J. Obst. & Gynec.*, **46**, 146, 1943.
107. HUFFORD, ALVAN, RAY. *J.A.M.A.*, **123**, 259, 1941.
108. ROBERTS, H. K., LOEFFEL, E., and MACBRYDE, C. M. *J.A.M.A.*, **123**, 261, 1943.
109. CLAUBERG, C. *Ztbl. f. Gynäck.*, **62**, 1034, 1939.
110. GREENE, R. *Brit. Med. J.*, **1**, 9, 1946.
111. FINKLER, R. S., and BECKER, S. *Am. J. Obst. & Gynec.*, **53**, 513, 1947.
112. GREENBLATT, R. B. "Office Endocrinology", Chas. C. Thomas, Springfield, Illinois, 1947.
113. JEFFCOTE, T. N. A. *Lancet*, **1**, 1045, 1940; *J. Obst. & Gynec. Brit. Emp.*, **45**, 893, 1938.
114. REDDOCH, J. W., and WIENER, W. B. *Am. J. Obst. & Gynec.*, **45**, 343, 1943.

115. SIKKEMA, S. H., and SEVERINGHAUS, E. L. *The Am. J. of Med.*, **2**, 251, 1947.
116. FLUHMAN, C. F. *J.A.M.A.*, **125**, 1, 1944.
117. ABARBANEL, A. R., ARANOW, H., and GOODFRIEND, M. J. *J.A.M.A.*, **121**, 1023, 1943.
118. HAMBLIN, E. C. "Endocrinology of Women", Chas. C. Thomas, Springfield, Illinois, 1945.
119. WILKINS, L., and FLEISCHMAN, W. *J. Clin. Endocrinol.*, **4**, 306, 1944.
120. REIMANN-HUNZIKER, G. *Peaxis*, Bern, **35**, 449, 1946.
121. COX, H. T. *Brit. Med. J.*, 4466, 191, 1946.
122. HAMBLIN, E. C., and DAVIS, C. D. *Am. J. Obst. & Gynec.*, **50**, 137, 1945.
123. ZONDEK, B. *J.A.M.A.*, **118**, 105, 1942.
124. FINKLER, R. S. *Am. J. Obst. & Gynec.*, **48**, 26, 1944.
125. HECKEL, M. J. *J. Clin. Endocrinol.*, **4**, 166, 1944.
126. KARNAKY, K. J. *South. Med. J.*, **37**, 510, 1944.
127. WEIJTLANDT, J. A. *J. Belge d'Urologie*, Brussels, **14**, 375, 1946.
128. HUGGINS, C., and McDONALD, D. F. *J. Clin. Endocrinol.*, **5**, 226, 1945.
129. GREENBLATT, R. B., and HAIR, L. Q. *J. Clin. Endocrinol.*, **2**, 315, 1942.
130. MCCREA, L. E. *J. Urol.*, **56**, 697, 1946.
131. KARNAKY, K. J. *West. J. Surg.*, **52**, 507, 1944.
132. KARNAKY, K. J. *J. Clin. Endocrinol.*, **3**, 413, 1943.
133. LYON, R. A. *Surg. Gynec. & Obst.*, **77**, 657, 1943.
134. GERBER, P. *Pennsylvania Med. J.*, **49**, 744, 1946.
135. SALMON, U. J., and GEIST, S. H. *J. Clin. Endocrinol.*, **3**, 235, 1943.
136. GASSNER, F. X. Twenty-Eighth Ann. Meet. of Assn. for Study of Internal Secretion, 1946.
137. HELLER, C. G., CHANDLER, R. E., and GORDON, B. M. *J. Clin. Endocrinol.*, **4**, 109, 1944.
138. ERSNER, J. A., MANN, B., and ZAMOSTEIN, B. *J. Clin. Endocrinol.*, **4**, 147, 1944.
139. FREED, S. C., EISIN, W. M., and GREENHILL, J. P. *J. Clin. Endocrinol.*, **3**, 89, 1943.
140. FERGUSSON J. D. *Lancet* **2**, 551, 1946.
141. PLANK, E. H. *South. Med. J.*, **39**, 794, 1946.
142. LUBIN, I. *Am. J. Obst. & Gynec.*, **51**, 225, 1946.
143. KONEFF, A. A., SIMPSON, M. E., and EVANS, H. M. *Anat. Rec.*, **94**, 167, 1946.
144. PINTO, R. M. *Rev. Soc. argent. de biol.*, **21**, 136, 1945.
145. BICKERS, W. *Am. J. Obst. & Gynec.*, **51**, 100, 1946.
146. MCGAVACK, T. H., and REINSTEIN, H. Twenty-Eighth Ann. Meet. of Assn. for Study of Internal Secretions, 1946.
147. SOLMON, V. J., WALKER, R. J., and GEIST, S. H. *Proc. Soc. Exper. Biol. & Med.*, **40**, 252, 1939.
148. DAVIDOFF, E., and REIFENSTEIN, E. C., JR., and GOODSTONE, G. L. *Am. J. Psychiat.*, **51**, 462, 1944.
149. GIBSON, R. *J. Ment. Sc.*, **89**, 278, 1943.



150. DANZIGER, L. *Arch. Neur. & Psychiat.*, **51**, 462, 1944.
151. RICHMAN, M. J., and ABARBANEL, A. R. *J. Clin. Endocrinol.*, **3**, 224, 1943.
152. BUXTON, C. L. *Am. J. Obst. & Gynec.*, **44**, 109, 1942.
153. KLAFTEN, E. *J. Clin. Endocrinol.*, **3**, 218, 1943.
154. WALKER, TAYLOR, C. *J. Clin. Endocrinol.*, **2**, 560, 1942.
155. HUTTON, E. L., and REISS, M. *J. Ment. Sc.*, **88**, 550, 1944.
156. WIESBADER, S. H., and FILLER, W. *Am. J. Obst. & Gynec.*, **51**, 75, 1946.
157. *Proc. Roy. Soc. Med. Rad. Sect.*, London, June 16, 1943.
158. HERRMANN, J. B., ADAIR, F. E., and WOODWARD, H. Q. *Arch. of Surg.*, **54**, 1, 1947.
159. FERGUSON, J. D. *Lancet*, **246**, 595, 1944.
160. SMITH, O. W., and SMITH, G. VAN S. *J. Clin. Endocrinol.*, **6**, 483, 1946.
161. KAISER *quoted by* REYNOLDS, S. R. M., presented at the Section on Obst. and Gynec. at the Ninety-Sixth Annual Session of the Am. Med. Assn., 1947.
162. SMITH, G. V., SMITH, O. W., and SCHILLER, S. *Am. J. Obst. & Gynec.*, **44**, 606, 1942.
163. *Ibid.*, **45**, 15, 1943.
164. HISAW, F. L., and GREEP, R. O. *Endocrinol.*, **23**, 1, 1938.
165. SEGALOFF, A. *Endocrinol.*, **38**, 212, 1946.
166. DRILL, V. A., and PFEIFFER, C. A. *Endocrinol.*, **38**, 300, 1946.
167. ZONDEK, B., and FINKELSTEIN, M. *Science*, 2723, 259, 1947.
168. HERTZ, R. *Endocrinol.*, **37**, 1, 1945.
169. KOREF, O., and ENGEL, P. *Endocrinol.*, **38**, 133, 1946.
170. HELLBAUM, A. A., and GREEP, R. O. *Proc. Soc. Exper. Biol. & Med.*, **63**, 53, 1946.

## PROGESTOGENS

## DEFINITION

THE term progestogen is applied to any substance possessing progestational activity.

**Progestin**

The name "Progestin" was originally given (Corner and Allen) to crude corpus luteum extracts with progestational activity. It is now used to describe the secretion product of the corpus luteum. Progestin is produced by the lutein cells of the corpus luteum, and by the adrenal cortex which, in addition, produces 17-hydroxy-progesterone. During pregnancy, progestin is secreted also by the chorio-placental system.

**Progesterone**

This is the purified crystalline product of the luteal hormone. It has been synthesized from pregnanediol, the excretion product of progestin in the urine of pregnant women, from stigmasterol, and through oxidation of cholesterol.

**Ethinyl Testosterone**

This compound, also known as ethisterone, pregneninolone, pregneninon-3-ol-17 and anhydro-hydroxy-progesterone, synthesized from oestradiol, is progestationally active when given by mouth [1].

## PHYSIOLOGY

Progestin, produced by the lutein cells of the corpus luteum, appears only during the second half of the menstrual cycle. It is suggested that the oestrogen peak occurring at about the midcycle causes increased release of LH from the anterior pituitary lobe, which induces corpus-luteum formation with progestin secretion. Progestin in turn stimulates the eosinophils to elaborate additional luteinizing hormone. Under the action of this hormone, increasing amounts of progestin are produced which, in addition to the eosinophils, stimulate basophilic activity with release of FSH, causing maturation of new follicles with renewed oestrogenic activity and regression of the corpus luteum.

During pregnancy, progestin is secreted in increasing amounts, reaching a peak in the eighth month. Studies of pregnanediol excretion have shown that relatively small amounts, rarely above 20-50 mg.

per 24 hours, are excreted during the first 3 months of pregnancy. Between the 30th and 90th days, a gradual rise occurs, which presumably marks the transfer of progestin secretion from the ovary to the placenta [2, 3].

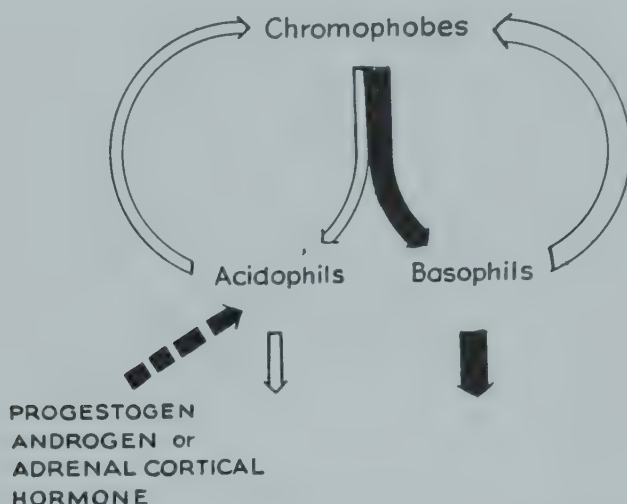


FIG. 12.—THE EFFECT OF INCREASED PROGESTOGEN, ANDROGEN OR ADRENAL CORTICAL HORMONE PRODUCTION ON THE FUNCTION OF THE ANTERIOR PITUITARY

It appears that in the animal progestin in small doses stimulates, whereas in large or continued doses it inhibits, pituitary gonadotrophic activity [5]. During pseudo-pregnancy in the rabbit, ovulation does not follow coitus because the persistent corpus luteum prevents

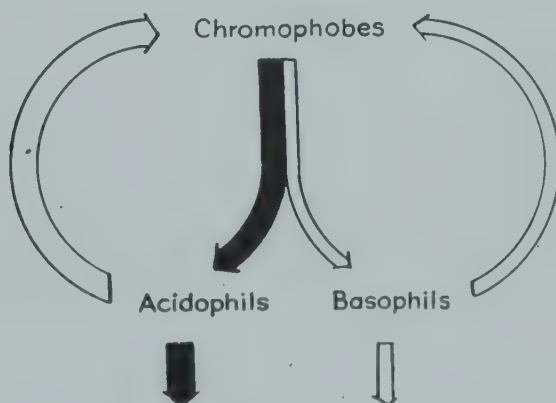


FIG. 13.—THE EFFECT OF PROGESTOGEN AND ADRENAL CORTICAL DEFICIENCY ON THE FUNCTION OF THE ANTERIOR PITUITARY

discharge of LH by the pituitary [6]. At the same time, the release of FSH which causes maturation of follicles is not inhibited [5].

The extirpation of the yellow body is followed by ovulation [7, 8, 9]. Similarly, removal of the corpus luteum in the cow on the



eighth day of the cycle induces ovulation 2 days later, whereas under normal conditions it occurs on the 20th–21st day. Progestin therapy in women with hyper-oestrogenic amenorrhoea, observed by temperature graphs, is often followed, after withdrawal of the progestin, by a fall in temperature below the pre-treatment level [10]. This is probably due to the fact that renewed release of LH from the anterior lobe, following the cessation of progestin activity in high doses, produces increased oestrogen secretion.

Eskin [5] observed increased post-coital gonadotrophic activity after one injection of 0.5 rabbit units of progestin into the rabbit. This finding was attributable to the pituitary-stimulating effect of progestin. Haemorrhagic spots in the ovaries, rarely seen after normal coitus, were observed in rabbits given pre-coital injections of progestin. The larger doses of 1.0 and 1.5 rabbit units failed, in most animals, to produce ovulation and haemorrhagic spots (see Chapters I and VII, and Figs. 3, 4, 6, 7, 12, 13).

### Progestogen Metabolism

Little is known about the metabolism of progestogen and the site of its inactivation. It appears that neither the ovary nor a functioning uterine mucosa is essential for the conversion of progestin into pregnanediol [11, 12]. Recent experimental evidence [13] showed that the liver played an important part in the inactivation of progestogen. In the partially hepatectomized rabbit only 25 mg. of progesterone administered by stomach tube were required to induce progestational changes comparable with those obtained with 200 mg. in the normal animal.

Pregnanediol is the end product of progestin metabolism, and is found combined with glycuronic acid to form the water-soluble compound sodium pregnanediol glycuronide. During the normal menstrual cycle it appears in the urine about 12 days before menstruation, in amounts of 1–5 mg. [14] and occasionally up to 11 mg. [15] per 24 hours' urine, disappearing 1–3 days before menstruation. A peak of excretion was noted about a week before the onset of the period. In pregnancy pregnanediol is usually excreted during the first 69 days, in amounts of 4–10 mg. daily [2], and rarely over 20 mg. daily [3]. Thereafter, the excretion value rises continuously up to a maximum of 105 mg. daily by the eighth month [2] (see Chapter VII).

EFFECTS OF PROGESTOGEN ON THE FEMALE  
REPRODUCTIVE ORGANS**Uterine Mucosa**

Progestin causes secretory changes in the glandular epithelium of the oestrogen-primed endometrium. According to Westman and Zondek [16, 17], these changes may occur in the endometrium even in anovulatory cycles, the progestin in such cases arising from the transformation of the granulosa cells into lutein cells by the action of the luteinizing hormone.

During the luteal phase, progestin causes a decline in uterine muscle tone, enhancing congestion of the endometrium. There is some evidence that progestin inhibits the motility of the uterine muscle, and inhibits the release of the posterior pituitary oxytocic factor. Cessation of progestin activity permits the release of the vasoconstrictor principle, which, acting on the oestrogen-sensitized uterine mucosa, produces constriction of the coiled arteries at their base adjacent to the uterine musculature.

Progestin prevents endometrial breakdown, but is unable to do so indefinitely except in the presence of small amounts of oestrogen [18]. Oestrogen, on the other hand, can postpone breakdown of the oestrogen-primed mucosa, but is unable to postpone bleeding when the mucosa is in the luteal phase, unless very large doses of oestrogen are administered at the time of corpus-luteum regression or progestogen withdrawal [19, 20].

During pregnancy, progestin prevents early abortion. Inhibition of uterine contractions, however, will not occur unless both progestin and oestrogen are present in the proper proportions [21].

The relationship of the uterus to progestin secretion is illustrated by the fact that the life of the corpora lutea is lengthened by removal of the uterus, and shortened by implantation of uterine tissue into hysterectomized-pseudopregnant rabbits. As the function of the uterus and the maintenance of the corpus luteum both require oestrogen, it is conceivable that the removal of the uterus may spare some available oestrogen and leave it at the disposal of the corpora lutea, thus prolonging their survival. The removal of the uterus and uterine contents, or extirpation of the placenta leaving the uterus intact, leads to a precipitous atrophy of the corpora lutea, while the implantation of the placenta in a hysterectomized-pregnant animal tends to postpone their regression. The factor concerned in regulating the corpus luteum under pregnant conditions appears to be present in the placental tissue; the uterus seems to play a less important part [39].

### **Fallopian Tubes**

Progestin stimulates secretory activity in the tubal mucosa but has an inhibitory effect on the tubal musculature.

### **Cervical Mucosa**

Progestin increases the alkalinity and permeability of the cervical mucosa [22].

### **Vagina**

The desquamation of cornified cells increases under the action of progestin.

### **Pelvic Ligaments**

Progestin relaxes the pelvic ligaments in the guinea pig. According to Hisaw [23], who prepared a pelvic-relaxing extract of cow's corpora lutea (Relaxine), the relaxing and progestational activities reside in separate factors [24].

### **Breasts**

Progestin induces growth of the lobule-alveolar system of the mammary gland.

## **THE RELATIONSHIP OF PROGESTIN TO THE ENDOCRINE GLANDS (see Figs. 1, 4, 12, 13)**

### **Anterior Pituitary**

Increased release of LH from the anterior lobe at about the mid-cycle induces corpus-luteum formation with progestin secretion. Progestin in turn, in moderate doses, presumably stimulates the eosinophils to release increased amounts of LH, which augment the secretion of progestin. It is suggested that larger doses of progestin further stimulate basophil activity with release of FSH. In the animal, large doses inhibit the release of LH (see Chapters I and VII).

### **Posterior Pituitary**

Progestin inhibits the release of the oxytocic factor.

### **Thyroid**

Progestin stimulates the thyroid gland (see Chapter III).

### **Adrenal Cortex**

Progesterone and 17-hydroxy-progesterone have been isolated from the adrenal cortex. Allen and Bourne [25] recently obtained an extract



of the whole adrenal gland, which is chemically distinct from the life-sustaining principle, and which induces luteinization and a considerable degree of endometrial hypertrophy. To this extract they gave the name adrenolutein. Large doses of desoxycorticosterone, one of the active compounds of the adrenal cortex, induces progestational changes in the endometrium of the rabbit and monkey [26, 27]. Pregnanediol, the chief product of progesterone metabolism, appears in unusually large amounts in the urine of patients with adrenal virilism [28, 29].

Protracted progesterone administration causes atrophy of the adrenal cortex.

### Ovaries

Progesterone inhibits ovulation and, in the non-pregnant animal, produces ovarian atrophy. It is suggested that this effect is mediated through the pituitary, progesterone inhibiting the release of LH from the anterior lobe [30]. Progesterone has no effect on follicle maturation (see Chapters I and VII).

### Oestrogens

Progesterone facilitates the conversion of oestrone to oestriol and to some extent protects oestrogen against inactivation [31], thus promoting the excretion of this substance [32]. Conversely, oestrogen augments the metabolism and utilization of progesterone when both hormones are given simultaneously in the presence of an oestrogen-primed uterus. On the organs of the reproductive system, progesterone and oestrogen exert opposite effects; thus, in the monkey progesterone produces blanching of the sex skin, 1.0 rabbit unit of progesterone inhibiting the sex-skin response to 300 rat units of oestrogen [33] (see Chapter VII).

### Androgens

Comparatively little is known about the relationship between progesterone and androgen. In spayed female opossums progesterone significantly decreases the weight and size of the reproductive tract (56 per cent), while testosterone propionate causes the weight and size of the tract to increase more than that of the untreated spayed control. Progesterone and testosterone propionate given simultaneously showed a synergistic effect and it appears that progesterone enhances the action of androgen [38]. This effect does not seem to exist in relation to the testis. The regeneration of atrophic testis in hypophysectomized rats accelerated by testosterone is inhibited by pregnenolone [4].

## STANDARDIZATION

*International Unit.*—This is the progestational activity of 1 mg. crystalline progesterone. The preparation to be tested is assayed by administering it for 5 days to an adult female rabbit, weighing approximately 600 grams and primed beforehand with 5–10 daily doses of oestrogen.

The progestational effect on the endometrium is evaluated microscopically.

Formerly used units are:

*Corner and Allen Unit*, approximately equal to the International Unit.

*The Clauberg Unit*, approximately equal to 0.6 International Units.

*The Clinical Unit* is about  $\frac{1}{3}$  of a Clauberg Unit.

PREPARATIONS FOR CLINICAL USE AND  
ADMINISTRATION**Progestin**

This is the natural non-crystalline extract of animal ovaries. Its dosage is expressed in biological units.

Administration: Intramuscular.

**Progesterone**

Progesterone is a crystalline synthetic product prepared from stigmasterol. Its dosage is expressed in milligrams or International Units.

Administration: Intramuscular injection or subcutaneous implantation. For implantation, pellets of pure crystalline progesterone are available in 50–150 mg. sizes.

Progesterone pellets, implanted subcutaneously, are absorbed at an average rate of about 20 per cent per month [34, 35]. This rate of absorption prevails during the first 3–4 months [36].

**Ethisterone**

(Ethinyl testosterone, Pregneninolone, Anhydrohydroxy-progesterone)

This is a synthetic crystalline product for oral use prepared from oestradiol. Its dosage is expressed in milligrams. The greatest effect is obtained by sublingual or sublabial absorption.

The ratio of sublingual to intramuscular doses is 4 or 5 to 1 in the rhesus monkey [37].

## CLINICAL APPLICATION AND APPROXIMATE DOSAGES

The progestogenic preparations are mainly employed for the prevention and treatment of habitual and threatened abortion. For the treatment of amenorrhoea and functional uterine bleeding due to ovarian deficiency, progestogens are usually given in a cyclic manner following the administration of oestrogens. In amenorrhoea associated with increased oestrogenic activity, progestogen is better given alone, producing uterine bleeding 2-3 days after cessation of treatment. Its use without preceding administration of oestrogens is contraindicated in amenorrhoea and in uterine bleeding due to marked oestrogen deficiency. Progestogens are effective in producing haemostasis in functional uterine bleeding, but their discontinuance is usually followed by withdrawal bleeding, a disadvantage of which short courses of oestrogens for haemostasis are usually free.

Progestogens for the relief of dysmenorrhoea, although frequently recommended, have on the whole proved very disappointing [50, 51, 52]. In view of recent evidence it is indeed doubtful whether their use in this condition is ever justifiable. Endometrial biopsies in 45 out of 53 patients suffering from dysmenorrhoea revealed a secretory pattern [46]. The production of dysmenorrhoeic pain by high doses of progestogen [39, 45], and the finding of protracted progestin activity in the presence of a low oestrogen level in those periods which are accompanied by dysmenorrhoea [10], indicates a shift in the oestrogen-progestogen ratio towards an absolute decrease of oestrogen as a cause of this symptom.

**Secondary Amenorrhoea due to Ovarian Deficiency**

Stilboestrol: 1.0 mg. orally daily for 10 days  
immediately followed by:

Ethisterone: 70 mg. orally daily for 5 days.

Spontaneous menstruation occurred after cessation of treatment (Cinberg [40]).

**Amenorrhoea due to Increased Oestrogen Activity**

Progesterone: 2.5 or 5 mg. linguets

or: Ethisterone: 5 mg. linguets.

Four to ten linguets daily for 3-10 days produce progestogen withdrawal bleeding (Greenblatt [41]).

The differentiation between hyper- and hypo-oestrogenic activity may be carried out by the vaginal smear technique. In hyper-oestro-



genism the vaginal smear consists of plaques of cornified epithelial cells, whereas in hypo-oestrogenism it shows basal epithelial cells, i.e. small round cells with large vesicular polychromatic nuclei [43].

### **Functional Uterine Bleeding due to Protracted Oestrogenic Action**

Progesterone: 10 mg. daily for 5 consecutive days.

Rubinstein [43].

or: Ethisterone: 30 mg. orally for 7 days during the first month.

20 mg. orally daily for 7 days, during the second and subsequent months.

Seegar Jones and Te Linde [44].

### **Metropathia Haemorrhagica**

Progesterone: 20 mg. on alternate days to a total of 4 doses every month, gradually reducing the dosage to 5–10 mg. each month.

This produced secretory endometria. Ethisterone, 50 mg. on alternate days for 4 doses, did not produce this result and was followed by a higher relapse rate (Scowen [42]).

### **Threatened and Habitual Abortion**

Progesterone: 1–5 mg. daily for the first 4 months of pregnancy.

In cases of cramps and bleedings, 5–10 mg. until symptoms subside; and then 1–5 mg. daily until the 4th month of pregnancy.

Scowen [42].

Some clinicians recommend the administration of oestrogen in addition to progestogen.

Ethisterone: 30–60 mg. orally daily

with: Diethylstilboestrol: 2–4 mg. orally daily until the foetus reaches viability (Hamblen [47]).

Vaux and Rakoff [48] advocate:

Progesterone: 10 mg.

and: Alpha-oestradiol benzoate: 10,000 I.B.U.

given together intramuscularly, from one syringe, 2 or 3 times weekly beginning at the fourth week of pregnancy and continuing to term.

The usual general measures should be applied in threatened abortion. It should be borne in mind that opiates, though they relieve

pain, are oxytocic. Habitual abortion should be investigated for the causative factors and treatment instituted accordingly.

### Acid Cervical Mucous

Ethisterone: 10 mg. orally twice daily throughout the cycle.  
Birnberg and others [22].

### Uterine Fibromyomas

Progesterone: 10 mg. 3-6 times a week.  
Goodman [51].

It appears that the beneficial effect of progesterone on fibroids produced in the guinea pig by continued oestrogen administrations [39] was successfully reproduced in the human by this dosage.

### BIBLIOGRAPHY

1. HÖLHWEG, W., and IMHOFFEN, H. H. *Klin. Wchnschr.*, **18**, 77, 1939.
2. BROWNE, J. S. Z., HENRY, J. S., and VENNING, E. H. *Am. J. Obst. & Gynec.*, **38**, 927, 1939.
3. JONES, G. E. S., DELFS, E., and STRAN, H. M. *Bull. Johns Hopkins Hosp.*, **75**, 359, 1944.
4. MASSON, G. *Am. J. Med. Sci.*, **212**, 1, 1946.
5. ESKIN, I. *Bull. Exper. Biol. & Med. U.S.S.R.*, **17**, 6, 52, 1944.
6. MAKEPEACE, A. W., WEINSTEIN, M. A., and FRIEDMAN, M. H. *Am. J. Physiol.*, **119**, 812, 1937.
7. HAMMOND, J. "The Physiology of Reproduction in the Cow", Macmillan Co., New York, 1927.
8. DRUMMOND-ROBINSON, G., and ASDELL, S. *Am. J. Physiol.*, **61**, 608, 1926.
9. PAPANICOLAOU, G. N. *J.A.M.A.*, **86**, 1422, 1926.
10. NIEBURGS, H. E. *J. Obst. & Gynec. Brit. Emp.*, **52**, 435, 1945.
11. BUXTON, C. L., and WESTPHAL, U. *Proc. Soc. Exper. Biol. & Med.*, **41**, 284, 1939.
12. HEARD, R. D. H., BAULD, W. S., and HOFFMAN, M. M. *J. Biol. Chem.*, **141**, 709, 1941.
13. MASSON, G., and HOFFMAN, M. M. *Endocrinol.*, **37**, 111, 1945.
14. BROWNE, J. S. L., and VENNING, E. H. *Endocrinol.*, **21**, 7111, 1937; *J. Clin. Invest.*, **16**, 678, 1937.
15. D'AMOUR, F. E. *J. Clin. Endocrinol.*, **3**, 41, 1943.
16. WESTMAN, A. *Arch. Gynec.*, **156**, 550, 1934.
17. ZONDEK, B. *Am. J. Obst. & Gynec.*, **33**, 979, 1937.
18. COURRIER, R. *Comptes Rend. Soc. Biol.*, **127**, 140, 1938.
19. HISAW, F. L., and GREEP, R. O. *Endocrinol.*, **23**, 1, 1938.
20. JILLMAN, J. S. *African J.M. Science*, **3**, 66, 1938.
21. HAMBLIN, E. C. *Am. J. Obst. & Gynec.*, **41**, 664, 1941.

22. BIRNBERG, C. H., KURZROK, L., and WEBER, H. *Am. J. Surg.*, **57**, 180, 1942.
23. HISAW, F. L. *Physiol.*, **2**, 59, 1929.
24. BROUHA, L. *Comptes Rend. Soc. Biol.*, **109**, 548, 1932.
25. ALLEN, R., and BOURNE, G. *Austral. J. Exper. Biol. & Med. Sci.*, **14**, 45, 1936.
26. MIESCHER, K., FISCHER, W. H., and TSCHOPP, E. *Nature*, **142**, 435, 1938.
27. ZUCKERMAN, S. *J. Endocrinol.*, **2**, 311, 1940.
28. BUTLER, G. C., and MARRIAN, G. F. *J. Biol. Chem.*, **119**, 565, 1938.
29. SALMON, U. J., GEIST, S. H., and SALMON, A. A. *Proc. Soc. Exper. Biol. & Med.*, **47**, 279, 1941.
30. SELYE, H. *Anat. Rec.*, **75**, 59, 1939.
31. PINCUS, G., and ZAHL, P. A. *J. Gen. Physiol.*, **20**, 879, 1937.
32. SMITH, G. V. S. and SMITH O. W. *Am. J. Physiol.*, **98**, 578, 1931.
33. HISAW, E. L., GREEP, R. O., and FEVOLD, H. L. *Am. J. Anat.*, **61**, 483, 1937.
34. WARWICK, N., and PARKER, A. S. *Lancet*, **1**, 406, 1940.
35. FORBES, *Endocrinol.*, **32**, 282, 1943.
36. GREENBLATT, R. B., and HAIR, Q. L. *J. Clin. Endocrinol.*, **5**, 38, 1945.
37. CORNER, G. W., JR. *Am. J. Obst. & Gynec.*, **47**, 670, 1944.
38. MORGAN, C. Twenty-Eighth Ann. Meet. of Assn. for Study of Internal Secretions, 1946.
39. IGLESIAS, R., and LIPSCHUTZ, A. *Lancet*, **251**, 488, 1946.
40. CINBERG, B. L. *J. Clin. Endocrinol.*, **3**, 167, 1943.
41. GREENBLATT, R. B. *J. Clin. Endocrinol.*, **4**, 156, 1944; *ibid.*, **4**, 321, 1944.
42. SCOWEN, E. F. *Proc. Roy. Soc. Med.*, **37**, 677, 1944.
43. RUBINSTEIN, B. *J. Clin. Endocrinol.*, **3**, 163, 1943.
44. SEEGAR JONES, G. E., and TE LINDE, R. W. *Bull. Johns Hopkins Hosp.* **71**, 5, 282, 1942.
45. FERIN, J. *Les Annales d'Endocrinologie*, **4**, 261, 1943.
46. GREENBLATT, R. B., MCCALL, E., and TORPIN, R. *Am. J. Obst. & Gynec.*, **42**, 50, 1941.
47. HAMBLIN, E. C. "Endocrinology of Women", Chas. C. Thomas, Springfield, Illinois, 1945.
48. VAUX, N. W., and RAKOFF, A. E. *Am. J. Obst. & Gynec.*, **50**, 353, 1945.
49. WHITE, P., and HAZEL HUNT. *J. Clin. Endocrinol.*, **3**, 500, 1943.
50. HOFFMAN, J. "Female Endocrinology", W. B. Saunders Co., Philadelphia, 1944.
51. GOODMAN, A. L. *J. Clin. Endocrinol.*, **6**, 402, 1946.
52. GREENBLATT, R. B. "Office Endocrinology", Chas. C. Thomas, Springfield, Illinois, 1947.



## ANDROGENS

THE male hormone is mainly produced by the interstitial cells of Leydig in the testes. Androgens are also elaborated by the adrenal cortex, and their secretion may increase abnormally in pathological conditions. Androgenic activity is estimated biologically by the output of urinary 17-ketosteroids. The androgens are closely related to oestrogens, since both have the same fundamental molecular structure of the cyclopentenophenanthrene molecule.

More than 40 androgenic steroids have been isolated and synthesized. The following are among the most important.

**Testosterone**

Testosterone is the active principle of testicular interstitial tissue in crystalline form. It has been synthesized from cholesterol by Ruzicka and others [1, 2].

**Androsterone**

Present in human male and female urine and synthesized from cholesterol by Ruzicka and co-workers [3].

**Dehydroandrosterone**

Extracted from human male urine by Butenandt and Danenbaum [4].

**Adrenosterone**

Extracted from bovine adrenals.

**Testosterone Propionate**

This is the most potent ester of testosterone, possessing 2–5 times the activity of testosterone.

**Methyltestosterone**

This is the methyl derivative of testosterone, and is active by the oral route.

Testosterone is 6–10 times as active as androsterone. Androsterone is more than twice as active as dehydroandrosterone.

## PHYSIOLOGY

**Testicular Function**

Spermatogenesis takes place in the seminal epithelium of the seminiferous tubules. These tubules contain mature but immotile sperma-

tozoa, motility being attained only in the epididymis. In the human, spermatogenesis starts at the age of puberty and is only slightly influenced by climatic conditions; in the majority of vertebrates, however, sperm production is seasonal.

The androgenic hormone, which is generally believed to be testosterone, is produced by the interstitial cells of the testis and the adrenal cortex. The amount of androgen secreted is measured by its excretion in the urine, where it is assayed either biologically by the comb growth of the capon or baby chick, or chemically by the reaction of 17-ketosteroids. Biological values reported vary between 30–100 I.U. daily [5, 6], and those for 17-ketosteroids between 8.1–22.6 mg. daily [7].

Testicular function is controlled by the anterior pituitary gland, spermatogenesis by the FSH and interstitial-cell production by the LH or interstitial-cell-stimulating hormone (ICSH). The ICSH is not a separate hormone but is identical with the LH hormone (Fevold and Evans).

Little is known about the inter-relationship of FSH and LH to the testicular hormone, or whether a cycle similar to that of the female occurs in the male. The hypothesis has been advanced that the germinal epithelium produces a hormone "Inhibin" which regulates pituitary activity.

Tornblom [8] demonstrated that testosterone administered in large amounts to castrated rats did not prevent the pituitary hypertrophy which occurs after castration. He concluded that it was not lack of testosterone which produced castrational changes in the pituitary. The application of X-rays to the testicles of rats in a dosage which sufficed to produce atrophy of the germinal epithelium without affecting the interstitial cell tissue, was, he observed, followed by castrational changes in the pituitary, showing that the pituitary inhibitory hormone resides in the germinal epithelium. He isolated from testicular tissue the fraction which prevented pituitary weight-increase in the castrate rat, and found that it possessed all the biological properties of oestradiol. It is interesting in this connection to note that increased quantities of oestrogen appear in the cock during the spring, the season of maximal testicular activity [9].

Evidence for the dual control of testicular function is supplied by Greep, Smith and others [10, 11], in their demonstration that the hypophysectomized male rat reacts to anterior pituitary FSH with tubular growth without changes in the accessory sex organs. The LH, on the other hand, produces hypertrophy of the interstitial cells and of all accessory sex organs. The pituitaries of normal male rats

contain follicle-stimulating hormone, while the blood serum of these animals contains luteinizing hormone and small amounts of follicle-stimulating hormone. Following castration, the pituitary LH as well as the FSH are markedly increased. The FSH in the blood-stream is also present in greater amounts, whereas the LH appears to be decreased. Treatment of castrated rats with testosterone propionate reduced the LH content of the pituitary but had little effect on the FSH. In the blood serum of these animals the amount of FSH was lowered, whereas the LH appeared to be increased after 15 and 30 days but not after 45 days of treatment [12].

### Spermatogenesis

#### *Inhibiting Action of Androgen*

A small dose (6 bird units) of testicular extract daily for 3 weeks inhibited testicular development in the young growing rat [13]. A larger dose (21 bird units) of bull testis had no deleterious effect [14, 15]. Testicular inhibition produced by testosterone propionate was greater the younger the animal, and small doses were more inhibitory than larger ones. Small doses of testosterone propionate (3 mg. weekly) given to pre-pubertal rats had an inhibitory effect on the testis, while no such effect was observed in post-pubertal animals. Larger doses (30 mg. weekly for 30 days) had a stimulating effect on spermatogenesis, in both the pre-pubertal and the post-pubertal animals. The same dose, however, given continuously, inhibited testicular function [16].

Waineman and co-workers [17], using testosterone propionate daily in the post-pubertal rat, observed weight reduction of the testes. Inhibition, however, was greater with small doses (0.2 mg.) than with larger doses (0.5 mg.). Zuckerman [18] used testosterone propionate in varying doses on 8 immature *Rhesus macaques* without effect on spermatogenesis.

Heckel [19] found that testosterone propionate in man inhibited spermatogenesis. Azoospermia produced by androgen like that produced by oestrogen is only temporary, however, for after withdrawal of the hormone testicular function returns to normal [20]. A case of deficient spermatogenesis treated by Hotchkiss [21] with small doses of testosterone propionate (100 mg. for 28 consecutive days) showed definite inhibition of spermatogenesis. Azoospermia, however, was transient and spontaneous recovery ensued. Very large doses (250-1,000 mg.) on 4 consecutive days, administered in 4 courses up to a total of 10,000 mg. over a period of 10 months, produced no inhibition to spermatogenesis.



### *Stimulating Action of Androgen*

Testosterone propionate or dehydroxysterone acetate, 3 mg. daily for 23 days, given to the immature rat did not inhibit spermatogenesis but produced atrophy of the interstitial cells [22]. Shay [16] found that testosterone propionate in large doses (30 mg. weekly for 30 days) stimulated spermatogenesis in both the pre-pubertal and post-pubertal animal, as long as administration of the hormone was not continued for a long time. Selye and Friedman [23] reported stimulation of testicular function by large and inhibition by small doses. Rubinstein and Kurland [24, 34], on the other hand, found that small doses increased and large doses inhibited testicular function in the pre-pubertal animal. Inhibition was not observed, however, in the post-pubertal animal.

Wells and Moore [25] produced spermatogenesis by administration of androgens in the non-breeding season. Soifer [26] found that spermatogenesis increased in the infertile man on administration of testosterone propionate in small doses (5–25 mg. twice weekly). Heckel and McCullagh [19, 27] observed that testosterone propionate, injected in doses approximating to 75 mg. per week over a period of 2 or 3 months, depressed the sperm count in man, sometimes to very low levels. When semen examinations were repeated after cessation of treatment, however, the sperm counts rose again to the original level or above it. Similar results followed the oral administration of methyl testosterone [28].

### *Direct Stimulation of Spermatogenesis*

There is some evidence that androgens may directly stimulate testicular function without the participation of the pituitary gland. Androgens given immediately after hypophysectomy in the guinea pig maintain the weight of the testes for 10 days but do not sustain spermatogenesis. Administration of androgens after hypophysectomy in the rat prevents atrophy of the seminiferous tubules but fails to protect the interstitial cells.

Nelson [29] showed the effect of combined androgen and adrenal cortical hormone administration in restoring spermatogenesis weeks after the removal of the pituitary gland. Very small doses of testosterone administered intra-testicularly to hypophysectomized rats served to sustain spermatogenesis locally [30].

Chu and You [135] obtained full spermatogenesis in the atrophic testes of hypophysectomized pigeons with testosterone propionate.

This gonadotrophic action of androgen does not appear in the presence of pituitary tissue, when the gonadal functions may be actually suppressed by such large doses. This suggests an antagonism between androgen and pituitary gonadotrophin. Preliminary experiments in which two crude pituitary extracts were treated with picric acid to inactivate the follicle-stimulating activity [136], or with trypsin to destroy the luteinizing activity [134], showed that both were capable of partially restoring the spermatogenic activity of the atrophic testes in hypophysectomized pigeons, and there was some suggestion that the extracts treated with picric acid which should possess luteinizing activity were more active in this respect than the extracts that underwent tryptic digestion.

The hypothesis is advanced that in male animals the pituitary luteinizing gonadotrophin stimulates the Leydig cells to secrete androgen, which in turn inhibits the liberation of further gonadotrophin while stimulating the germinal epithelium of the testis. As the concentration of androgen diminishes so the secretion of pituitary gonadotrophin is restored and so on.

#### OESTROGENIC EFFECT OF ANDROGENS

Testosterone in the infant rodent produces oestrus and vaginal introitus [31, 32, 33], and postpones oestrogen-withdrawal bleeding in the castrated rhesus monkey. Paschkis and co-workers [35] report that androsterone and testosterone administration to the dog was followed by oestrogen excretion. Hartman [36] noted that testosterone invariably had an oestrogenic action on the sex-skin of the female monkey. After administration of testosterone, the sex-skin was always a brilliant red. In this respect he found progesterone antagonistic to oestrogen. Testosterone did not antagonize the action of oestrogen. Androgens and oestrogens, when given simultaneously in moderate doses, act synergically on both the smooth muscle and epithelium of the seminal vesicles [37].

In the human, testosterone prevents breast engorgement and the onset of lactation, exerting the same effect as oestrogen [38, 39]. The usual atrophy of the uterus which follows extirpation of the ovaries can be prevented by testosterone [40]. McKeown and others [41, 42], however, hold that testosterone administration does not produce enlargement of the uterus in the ovariectomized rabbit.

Nathanson and Towne [43] noted a profuse vaginal discharge after administration of testosterone. The discharge consisted of cornified cells, and the vaginal smear was of the oestrogenic type. Salmon [44] obtained an oestrogenic type of smear only after administration



of concentrated doses (50–100 mg. per day) of testosterone propionate. Twenty-five mg. daily did not produce this effect. Implantations of testosterone propionate up to 400 mg. in women, for the treatment of various functional disorders, did not interfere with development of the progestational endometrium or with glycogen deposition in the superficial layers of the vaginal mucosa. In menopausal women with initial smears of the castrate type, larger doses (400 mg.) of testosterone appeared to have an oestrogenic effect [45].

Nation and others [46] reported three cases of interstitial cell tumour of the testis associated with gynecomastia.

#### MECHANISM OF ANDROGENIC ACTION

On the basis of the evidence cited, it may be concluded that the effect of androgens on spermatogenesis depends on dosage, length of treatment, and age of the subject. Small doses may inhibit spermatogenesis, and do in fact nearly always inhibit testicular development in the experimental pre-pubertal animal. Selye [47] attributes the inhibitory effect of small doses of testosterone on testicular function to the fact that in such amounts testosterone is oestrogenically active. Large doses of testosterone stimulate spermatogenesis and can even counteract the anti-spermatogenetic effect of oestradiol [48]. The highest oestrogenic effect of androgens was found to be exerted by testosterone and methyl testosterone [49].

Thus, granted that the inhibiting effect on spermatogenesis is mediated through the pituitary, it is reasonable to assume that androgens in small (oestrogenic) doses act by inhibition of FSH, thus depriving the seminal epithelium of the necessary stimulus for spermatogenesis.

With the cessation of treatment, spermatogenesis returns to the normal or even higher levels. Thus it appears that the temporary suppression of pituitary FSH elicits greater pituitary activity later. This is analogous to the "release phenomenon" described by Engle and others [50, 51], according to which cessation of strong oestrogenic activity induces hypophyseal activity after its temporary suppression. Supporting evidence that oestrogens may increase spermatogenesis [52, 53] has recently been supplied.

On the other hand, there is a growing body of evidence that oestrogens, particularly in small amounts, have a stimulating effect on the pituitary [50, 54–60] unless their administration is continued for a very long period (see Chapter VII). In view of this, the hypothesis is advanced that small doses of androgens stimulate the eosinophils to produce more LH, this being followed by a decrease in basophil-cell



activity with reduced production of FSH. This would explain the decreased spermatogenesis in the male, just as the increased release of the LH would explain the oestrogenic effect in the female.

Large doses of androgens have no inhibitory effect on spermatogenesis even in the pre-pubertal animal. Treatment often results in increased spermatogenesis probably due to inhibition of LH and increased release of FSH.

Like oestrogens, androgenic substances may cause atrophy of the ovary, inhibition of ovulation, and regression of corpus luteum, as demonstrated in the experimental animal by Zuckerman [61] and in man by Geist and Salmon [62]; or they may stimulate follicular maturation and cause luteinization. The different response obtained is probably due to a difference in dosage and duration of treatment.

### **Castration**

Castration before puberty causes delayed closure of the epiphyses, resulting in disproportionate lengthening of the limbs. Muscular development is poor, the distribution of hair and of subcutaneous fat conforms to the feminine type, the voice remains high-pitched, and all other secondary sex characters fail to develop. Castration after puberty is characterized by general loss of body hair, thinning of the beard, changes in the texture of the scalp hair which becomes finer and softer, and the development of a high-pitched feminine voice; muscular atrophy, and obesity of the feminine type may occur. Genital atrophy takes place with loss of libido and potency. Circulatory changes are manifested in the form of flushes like those commonly observed in menopausal women.

### **Pregnancy**

The treatment of pregnant animals with androgens produces intersexuality in the female offspring [63]. The freemartin twin, which is frequently seen in sheep and cattle, is supposed to be due to the influence of androgenic secretion of the male twin on the female partner *in utero*.

### **Carbohydrate Metabolism**

Opinions differ on whether or not androgens inhibit gluconeogenesis [64]. Prolonged administration of methyl testosterone causes a transient lowering of glucose tolerance and decrease in liver glycogen [65, 66, 67]. This effect is not obtained with testosterone propionate or in hypophysectomized or thyroidectomized animals [68].

### **Electrolyte and Water Metabolism**

Androgens promote sodium, chloride and water storage, which results in gain of body weight and in tissue hydration.

### **Haemopoiesis**

Androgens increase the number of red cells, the haemoglobin content, and the volume of packed cells in normal, senile and castrated male rats. Oestrogens have no such effect [69].

### **Growth**

Administration of androgens accelerates growth in young boys by three or four times the normal rate. Though androgens induce premature closure of epiphyses, they do not appear to affect the final height, because the rapid ageing of bone is counterbalanced by the rapid increase in height. In addition to osseous maturation, androgens influence the development of the musculature and every tissue in the body [70]. Calcium excretion is conspicuously lowered, and increased nitrogen retention favours formation of new protein. Blood-nitrogen values are not increased. The excretion of urea and creatine in the urine is decreased.

The healing of fractures is greatly aided by the administration of androgens [118, 133], though large doses apparently exert an inhibitory action on bone growth [117].

### THE RELATIONSHIP OF ANDROGENS TO THE ENDOCRINE GLANDS (*see Figs. 1, 12, 13*)

#### **Anterior Pituitary Gland**

The LH of the anterior lobe stimulates androgen secretion, small doses of which in turn presumably stimulate the pituitary to release more LH. Prolonged administration of androgens causes reduction of the eosinophilic cells and compensatory increase of the chromophobe cells [71]. Large doses thus inhibit growth and small doses enhance it by stimulating nitrogen retention, general metabolism [72, 73], and presumably increase of eosinophils. Low or retarded androgen secretion in the pre-pubertal boy is accompanied by overgrowth of the long bones, causing eunuchoidism.

#### **Posterior Pituitary Lobe (Oxytocic Factor)**

Testosterone propionate 10 mg. inhibits the action of the posterior pituitary lobe on the uterus of female rabbits [74].

### Thyroid

Androgens increase the basal metabolic rate. Injections of testosterone propionate 25–50 mg. 3 times a week increase the rate by about 30 per cent; or even by 60 per cent if given in conjunction with methyl testosterone [70].

### Parathyroid

In the male, hyperparathyroidism is frequently accompanied by loss of both potency and libido.

### Thymus

Androgens cause involution of the thymus. Castration results in its hypertrophy (see Chapter X).

### Adrenals

Adrenalectomy causes testicular atrophy, and chronic adrenal insufficiency is associated with impotence and loss of libido. Hyperfunction of the cortex, due to hyperplasia or tumour, has a virilizing effect in the female, and causes premature appearance of secondary sex characters in the male.

Injections of adrenotrophic extracts produce enlargement of the seminal vesicles and prostate in castrated rats, but only in the presence of the adrenal cortex [75]. In the male, castration produces hypertrophy of the adrenal cortex, which may be prevented by the administration of androgens [76]. The action of testosterone does not depend on intact adrenals [77]. In the presence of normal adrenals, prolonged action of androgens causes adrenal cortical atrophy. Administration of methyl testosterone reduces the excretion of 17-ketosteroids [78].

In the normal mechanism of sexual development, the adrenal cortex plays the part of a bisexual accessory sex gland, which is active throughout life and secretes both androgenic and oestrogenic hormones under the control of the pituitary [79]. In women, the occurrence, during early foetal life, of a short period of androgenic and heterosexual development of cortical origin, introduces an element of heterosexual instability, tending to intersexuality under conditions of adreno-pituitary imbalance. Heterosexual changes are far rarer in the male, in whom no comparable oestrogenic period occurs (see Chapter V).



### **Pancreas (Islet System)**

It is suggested that androgens act synergically with insulin, probably by reducing gluconeogenesis, owing to the inhibitory effect on the adrenal cortex.

### **Oestrogens**

Before the onset of adolescence, both sexes excrete approximately the same amounts of oestrogen in the urine [80]. During adult life, oestrogen excretion in the male is about one-third of that in the female [81]. The sites of oestrogen production in the male are the adrenal cortex and presumably the germinal epithelium of the testes. The excretion rate of oestrogen in the urine of normal men is between 90–120 I.U. daily [82], against 50 m.u. per litre in women during the follicular phase [83], and 1,000 I.U. per 24-hour urine at the peaks of oestrogen excretion [84]. The only oestrogen found in the male urine is oestrone [85].

The actions of oestrogen and androgen, in the male and in the female, are apparently synergic, as long as both exist in the ratio necessary to maintain the balance of pituitary activity. A significant alteration in the oestrogen/androgen ratio produces an antagonistic relationship of the hormones in both sexes. A shift to increased activity of the opposite sex hormone causes defeminization with heterosexual changes in the female, and demasculinization in the male. It appears reasonable to assume that a shift in the ratio towards an absolute or relative increase in the isosexual hormone may occur (see Chapter VII).

### **THE RELATIONSHIP OF ANDROGENS TO VITAMINS**

Vitamin A deficiency causes sloughing of the germinal cells and shrinking of the seminiferous tubules (see Chapter XIV).

Vitamin B deficiency may cause gonadal hypofunction owing to impaired liver function. Androgens are inactivated in the liver. Vitamin B complex deficiency does not significantly impair the inactivation of testosterone propionate in the liver of the male rat; but it impairs the inactivation of oestrogens and thus produces a serious alteration in the oestrogen-androgen equilibrium [86] (see Chapter XIV).

Vitamin E deficient diets cause testicular degeneration in rats (see Chapter XIV).

### Standardization

The International Unit is defined as the equivalent of 100 gamma (0.0001 grams) of crystalline androsterone.

The male hormone is assayed by its comb-growth-promoting action in the caponized Leghorn. The extract to be tested is injected into a muscle or directly into the base of the comb, or applied by inunction into the comb.

Another method for the assay of androgenic activity is in terms of the weight increase produced by the extract under examination in the prostate and seminal vesicles of the castrate rat or mouse.

The capacity of androgens to change the ivory-coloured bill of the English sparrow to black may be utilized as another method of assay.

### PREPARATIONS FOR CLINICAL USE AND ADMINISTRATION

#### Testosterone Propionate

5, 10, 25 mg. for intramuscular injection.

#### Methyl Testosterone

5 and 10 mg. tablets for sublingual or sublabial absorption.

#### Testosterone in Propylene Glycol-Alcohol

For sublingual use:

25 mg. per c.c. solution = 5 drops = 4-5 mg.

50 mg. per c.c. solution = 5 drops = 8-9 mg.

100 mg. per c.c. solution

25 drops of 50 mg. per c.c. is approximately equal to testosterone propionate 25 mg.

Hurxthal [87]

#### Testosterone in Aqueous Suspension .

An aqueous suspension of testosterone crystals is at least twice as active as the same material dissolved in oil. This increase in activity is probably the result of its slower absorption into the blood-stream [113]. The apparent period of effective supply of testosterone in aqueous suspension from each injection is from 4 to 7 days [111].

#### Implantations

Chemically pure crystalline testosterone or testosterone propionate can be obtained in the form of pellets weighing 25, 50, 75, 100 or 200 mg. Implantations of testosterone propionate are not subject to ghost form-

ation if cast rather than compressed pellets are used, since the former unlike the latter do not contain pores. The initial absorption rate of cast pellets is approximately 1.1 mg. per day for the first 50 days and afterwards approximately 0.34 mg. per day [88].

The crystalline hormone is about five times as effective when implanted in pellet form as it is when injected in oily solution; and need accordingly be given in only about a fifth of the dose (Hamilton).

### *Method of Implantation*

(See p. 148.)

## CLINICAL APPLICATIONS AND APPROXIMATE DOSAGES IN MEN

### **Delayed Puberty**

Testosterone propionate: 15–50 mg. intramuscularly on alternate days.

Morgan [91]

### **Eunuchoidism**

Testosterone propionate: 20–50 mg. 3 times a week.

or: Methyl testosterone: 30–90 mg. daily.

or: Testosterone propionate: Pellets implanted to a total of about 100 mg.

Thompson [70].

### **Eunuchoidism in Adults**

Testosterone propionate: 100–200 mg. weekly or more often.

or: Testosterone propionate: Pellets implanted into the subcutaneous tissue, either in the interscapular region or in the anterior abdominal wall. 50 mg. pellets are absorbed in about 8–10 weeks.

### **Hypogonadism**

Testosterone: 150-mg. pellets implanted in the thigh (the effect lasts for 2 months).

Testosterone propionate: 50 mg. per week.

Testosterone propionate 25 mg. a week was in one patient satisfactorily replaced by 4 drops of a 50 mg. per c.c. solution = 6 mg. daily or 42 mg. weekly.

Hurxthal [87].



Methyl testosterone linguets: 15 mg. daily for sublingual absorption.

Lisser and Curtis [93]

Testosterone crystals in aqueous suspension: 20 mg. intramuscularly every 4-7 days.

Severinghaus and Sikkema [111].

### **Male Climacteric**

Testosterone propionate: 25 mg. intramuscularly 3 times a week for 2-3 months.

Werner and others [97, 70].

### **Geriatric Fatiguability**

Methyl testosterone: 30-40 mg. daily.

Simonson, Kearne and Enzer [98].

### **Senile Pruritus in the Male**

Testosterone propionate: 20-mg. pellet implanted.

Feldman, Pollock and Abarbanel [99].

Testosterone propionate, 10 mg. daily, and Testosterone propionate ointment produced favourable results.

Dobes, Jones and Franks [110].

### **Homosexuality**

Either testosterone propionate: or methyl testosterone may be used. In one series of 11 cases in which they were given for 3-4 months, beneficial results were recorded in 3 only. Five of the patients reported an intensification of the homosexual drive and no results were obtained in the remaining 3.

Glass and Johnson [102].

### **GENERAL THERAPEUTIC USES**

The employment of androgens for other than substitution therapy has given somewhat conflicting results, and evaluation of their pharmacological effects in diseases not directly due to androgen deficiency must await further investigation. A short non-critical survey is given of those conditions which have apparently benefited from androgen therapy.

**Hypertension (Moderate)**

Testosterone propionate: 10 mg. 3 times a week. Total dosage 50-200 mg.

Marques [103].

**Angina-like Pain accompanying the Male Climacteric**

Testosterone propionate: 25 mg. intramuscularly or 300-mg. pellet implants.

McGavach [106].

**Angina Pectoris**

Testosterone propionate: 25 mg. every second to fifth day for a total of 2-25 injections.

Improvement was noted after an average of 28 days' treatment.

Lesser [107, 105].

Testosterone propionate: 25 mg. three times a week.

Results are questionable.

Levine [108].

Testosterone propionate: 25 mg. 3 times a week for 1 month or longer.

Improvement was noted after an average of 4 injections.

Hamm [109].

**Macrocytic Anaemia**

Testosterone propionate: 10 mg. twice weekly.

or: Methyl testosterone: 20 mg. daily for 10 days.

Thyroid: 2 grams daily was administered as well.

This treatment was followed by a brisk rise in the blood count.

Glass [112].

Testosterone propionate: 25 mg. twice weekly.

This exerts a stimulating effect on the bone marrow and is indicated in microcytic and macrocytic anaemia complicating long-standing hypogonadism or hypopituitarism.

Watkinson, McMenemey and Evans [89].

### Enuresis

Methyl testosterone: 10–20 mg. daily.

or: Testosterone: Daily local application of 2 doses of 4 mg. each.  
Schultz and Anderson [114].

Methyl testosterone: 10–30 mg. daily for 1–3 months.

If no significant improvement was obtained within a fortnight, testosterone propionate 10 mg. weekly was given for 3–15 weeks, in which time 59 or 75 children were cured and 10 children improved.

Kugelmass [104].

### Progressive Chronic Deafness (Otosclerosis)

Testosterone propionate: 10 mg. intramuscularly.

12 Injections resulted in improvement in 54 per cent of cases.

Prager [115].

### Bronchial Asthma associated with Impotence

Testosterone propionate: 25 mg. 3 times weekly.

(The Cleveland Clinic).

### Cushing's Syndrome

Testosterone propionate: 25-mg. daily injections caused diminution in nitrogen excretion and disappearance of creatinuria.

Methyl testosterone: 40 mg. daily by mouth caused striking decrease in nitrogen excretion and great increase in creatine excretion. No change in creatinine excretion occurred.

Testosterone: 40 mg. daily did not produce unequivocal changes in excretion of nitrogen, creatine or creatinine.

Deakins, Friedgood and Ferrebee [116].

### Addison's Disease

Testosterone propionate: 50 mg. daily

or: Methyl testosterone: 90 mg. daily.

Administration of desoxycorticosterone and sodium chloride also formed part of the treatment which was followed by gain in body-weight, fall in the urine excretion of nitrogen, potassium and sodium, and marked fall in the serum potassium concentration [64].



**Hypophyseal Cachexia (Simmonds's Disease)**

Methyl testosterone: 20 mg. 5 times daily.

This, after several weeks of treatment, was followed by increasing signs of well-being, greater muscular strength, increase in appetite and return of libido, accompanied by nitrogen retention, increase in creatinuria, increase in basal metabolic rate, and decrease in serum cholesterol.

Werner and West [119].

Escamillo, Roberts and Lisser [120].

Lisser and Curtis [101].

**Thyrotoxicosis**

Testosterone propionate: 12.5 mg. daily.

This decreases the hypercreatinuria characteristic of thyrotoxicosis and improves the clinical state of the patient.

Methyl testosterone, on the other hand, increases the hypercreatinuria and aggravates the thyrotoxic condition.

Kinsell and co-workers [121].

**Premature Infants**

Methyl testosterone: 2.5 mg. in feedings every 12 hours over a period of from 4 to 7 weeks.

All 15 infants weighing less than 2,000 grams with an approximate 50 per cent prediction of mortality survived.

Shelton and Varden [100].

**Bone Fractures**

Testosterone propionate: 25 mg. daily 3 times a week.

Davis [133].

**IN WOMAN**

Opinions are sharply divided on the use of androgens for the treatment of endocrine disorders involving the reproductive system. Hamblen strictly opposes the procedure as unphysiological, while others advocate it for nearly the whole range of menstrual disorders. Although many disorders undoubtedly benefit from androgenic treatment, its rationale is still a matter of speculation. It seems reasonable to assume that the results must be viewed against the background of pituitary physiology, with particular reference to the effect of androgens on the anterior lobe, which in turn depends on the dosage

employed and length of treatment. In most cases, a similar pituitary effect, accompanied by improvement of the disorder, can be obtained by more physiological procedures, e.g. by the application of oestrogens or in cyclic oestrogen-progestogen treatment.

Relatively large amounts of the heterosexual hormone are excreted in woman throughout life, and a shift in the oestrogen-androgen equilibrium towards an increase in the male hormone produces a variety of disorders in the endocrine system associated with abnormal menstruation and mental changes. Similar disorders, though of different nature, occur when the hormonal balance is shifted towards an increase of oestrogens. Further investigation of this as yet little understood condition, which often causes menstrual disorders sometimes identical with those observed in oestrogen deficiency, may indicate the place of androgens in the treatment of female endocrine disorders, particularly in cases in which excessive oestrogen activity is relative and due to a decrease of androgens.

The present data on the use of androgens in woman do not reveal whether the disorders which benefit are associated with a shift in the oestrogen-androgen ratio. Androgens have been employed by intramuscular injection or implantation of testosterone or testosterone propionate, or by oral administration of methyl testosterone. Moderate doses below 300 mg. per month do not apparently produce masculinizing effects—e.g. testosterone propionate 10–25 mg. once to three times a week, or methyl testosterone 5–10 mg. daily over short periods. Greenblatt and co-workers [45] found that masculinizing symptoms were absent when they used implants of 25–400 mg. testosterone propionate. Arrhenomimetic effects of androgen became, however, apparent when used in doses of about 150 mg. weekly for 10 months [132].

The following is a non-critical review of some androgen treatment schedules in female disorders.

### **Functional Uterine Bleeding**

Testosterone: Implantation of 96–205 mg. pellets

Greenblatt [122].

### **Functional Uterine Bleeding associated with Fibroids**

Testosterone propionate: Implantation of 25–302 mg. pellets.

Greenblatt [122].

**Functional Menorrhagia**

Testosterone propionate: 25 mg. 2-3 times weekly.  
Salmon and Geist [123].

Testosterone propionate: 25 mg. daily  
and: Progesterone: 10 mg. daily  
for 4-5 days. This results in a rapid cessation of bleeding  
which, after an interval of 4-5 days, is followed by a short  
bout of withdrawal bleeding.  
Greenblatt and Kupperman [96].

**Intracyclic Bleeding**

Testosterone propionate: 10-25 mg. at weekly intervals.  
Methyl testosterone: 5-10 mg. throughout the intermenstrual  
period.  
Greenblatt [124].

**Dysmenorrhoea**

Testosterone propionate: Implantations of 150-400 mg. re-  
sulted in some relief of pain.  
Greenblatt [122].  
Testosterone propionate: 100-200 mg. during the last 2 weeks  
of the cycle.  
Geist and Salmon [123]

**Premenstrual Tension**

Testosterone propionate: 25 mg. 2-3 times weekly beginning  
on the tenth to fifteenth day of the cycle and continuing  
till 2 or 3 days before menstruation.  
Geist and Salmon [123].

Methyl testosterone: 10 mg. daily starting 10-7 days before the  
expected period

or: Testosterone in aqueous suspension: 20 mg. injections.  
Freed [95, 113].

**Premenstrual Mastopathia**

Testosterone propionate: 10 mg. twice weekly beginning on the  
tenth day of the cycle and continuing for 3 cycles, reducing  
the dose by 25-50 per cent each month.  
Geist and Salmon [123].



### **Painful Haemorrhagic Ovulation**

Testosterone propionate: 25 mg. on alternate days for 4-6 doses on the second to fifth days of the cycle.

Geist and Salmon [123].

### **Uterine Fibroids**

In cases with no menstrual disturbances but with pelvic discomfort or debility, administration of testosterone propionate was followed by shrinking of the palpable tumour.

Greenblatt [124].

### **Endometriosis**

Testosterone propionate: 25-150 mg. every third day.

Hirst [125, 94].

### **Pre-operative Treatment of Endometriosis**

Testosterone propionate: 25 mg. intramuscularly twice weekly.

Miller [126].

### **Decreased Libido**

Testosterone propionate: Implantation of 25-400 mg. pellet.

No virilizing symptoms have been noted.

Greenblatt and others [45].

Testosterone propionate: 10-25 mg. 3 times a week.

or: Methyl testosterone: 10-30 mg. daily

or: Testosterone pellets: 25 mg. 2-8 pellets.

Salmon and Geist [127].

### **Nymphomania**

Testosterone propionate: 25 mg. at varying intervals.

Rubinstein, Shapiro and Freeman [128].

### **Dyspareunia**

This condition, when due to dryness of the vagina, was partly alleviated by the mucoid vaginal secretion following androgen treatment.

Greenblatt [122].

### **Post-partum Engorgement of the Breasts**

Testosterone propionate: 50 mg. daily for 3 days beginning immediately after delivery.

Geist and Salmon [123].

Methyl testosterone: 30 mg. every 4 hours for 5 doses.

Duckman and Trurino [129].

### **Mammary Cancer**

Testosterone propionate: 5–25 mg. 1–3 times a week for 10–12 doses.

This treatment resulted in decrease of pain in 50 per cent of patients.

Farrow and Woodward [130].

Testosterone propionate: 25 mg. every other day up to a total dose of 1,550 mg.

This resulted in marked improvement, almost reaching a clinical cure, owing to an extraordinary proliferation of the fibrous tissue which impeded and blocked the extension of the neoplastic cells and finally crushed them.

Fels [131].

Testosterone propionate: 200 mg. daily.

Striking regression of the lesions occurred in one out of three patients treated.

Herrmann and Adair [92].

### **Carcinoma of the Cervix, Breast, Ovary, Fallopian Tubes, Uterine Corpus**

Testosterone propionate: 140–150 mg. weekly for 10 months.

This treatment produced symptomatic improvement only. There was no regression or even retardation of the malignant process. Masculinizing symptoms appeared in all patients.

Abel [132].

Wyatt [90].

Repeated serum calcium studies are required to recognize and prevent hypercalcaemia.

## BIBLIOGRAPHY

1. RUZICKA, L., and WEINSTEIN, A. *Helv. chim. Acta.*, **18**, 1264, 1935.
2. BUTENANDT, A., and HAMISCH, G. *Ztschr. f. physiol. Chem.*, **237**, 89, 1935.
3. RUZICKA, L., GOLDBERG, M. W., MEYER, J., BRUEGGER, H., and EICHENBERGER, E. *Helv. chim. Acta.*, **17**, 1395, 1934.
4. BUTENANDT, A., and DANENBAUM, H. *Ztschr. f. physiol. Chem.*, **229**, 192, 1934.
5. GALLAGHER, T. F., PETERSON, D. H., DORFMAN, R. T., KENYON, A. T., and KOCH, F. G. *J. Clin. Invest.*, **16**, 695, 1937.
6. KOCH, F. C. *Biol. Symposia*, **9**, 41, 1942.
7. TALBOT, N. B., BUTLER, A. M., BERMAN, R. A., RODRIGUEZ, P. M., and MACLACHLAN, E. A. *Am. J. Dis. Child.*, **65**, 364, 1943.
8. TÖRNBLOM, "Uppsala Lak Foren", Haft 1, Och. 2, p. 1, 1942.
9. FALIN GRONTZEVA, K. E. *Bull. Exper. Biol. & Med. U.S.S.R.* **16**, 7-8, 68, 1943.
10. GREEP, R. O. *Anat. Rec.* 67 (Suppl.), 22, 1937.
11. SMITH, P. E., ENGLE, E. T., and TYNDALE, H. H. *Proc. Soc. Exper. Med. & Biol.*, **31**, 745, 1934.
12. HELLBAUM, A. A., and GREEP, R. A. *Endocrinol.*, **32**, 33, 1943.
13. MOORE, C. R., and PRICE, D. *Am. J. Anat.*, **50**, 13, 1932.
14. MOORE, C. R., and PRICE, D. *Anat. Rec.*, **71**, 59, 1938.
15. MOORE, C. R., and PRICE, D. *Endocrinol.*, **21**, 313, 1937.
16. SHAY, H., GERSHON-COHEN, J., PASCHKIS, K. E., and FELS, S. S. *Endocrinol.*, **28**, 485, 1941.
17. WAINMAN, P., REESE, J. D., and KONEFF, A. A. *Endocrinol.*, **31**, 303, 1942.
18. ZUCKERMAN, S. *Lancet*, **1**, 1162, 1938.
19. HECKEL, N. J. *Proc. Soc. Exper. Biol. & Med.*, **40**, 658, 1939.
20. HECKEL, N. J. *J. Clin. Endocrinol.*, **4**, 173, 1944.
21. HOTCHKISS. *J. Clin. Endocrinol.*, **4**, 117, 1944.
22. CUTULY, E., and CUTULY, E. C. *Endocrinol.*, **26**, 502, 1940.
23. SELYE, H., and FRIEDMAN, S. *Endocrinol.*, **28**, 129, 1941.
24. RUBINSTEIN, H. S., and KURLAND, A. A. *Endocrinol.*, **28**, 495, 1941.
25. WELLS, L. J., and MOORE, C. R. *Anat. Rec.*, **68**, 181, 1936.
26. SOIFER, S. *Urol. & Cut. Rev.*, **45**, 137, 1941.
27. MCCULLAGH, D. R. *J. Urol.*, **42**, 1265, 1939.
28. MCCULLAGH, E. P. *J. Clin. Endocrinol.*, **3**, 375, 1943.
29. NELSON, W. O. *Am. J. Physiol.*, **129**, 430, 1940.
30. DVOSKIN, S. *Proc. Soc. Exper. Biol. & Med.*, **54**, 111, 1943.
31. DEANESLEY, R., and PARKES, A. S. *Brit. Med. J.*, **1**, 257, 1936.
32. KORENCHEVSKY, V., DENNISON, M., and SIMPSON, S. L. *Biochem. J.*, **29**, 2534, 1935.
33. BUTENANDT, A., and KINDZUS, H. *Ztschr. f. physiol. Chem.*, **237**, 75, 1935.



34. RUBINSTEIN, H. S., and KURLAND, A. A. *South. Med. J.*, **32**, 499, 1939.
35. PASCHKIS, K. G., CANTAROW, A., RAKOFF, A. E., HANSEN, A. E., and WALKLING, A. A. *Proc. Soc. Exper. Biol. & Med.*, **33**, 234, 1943.
36. HARTMEN, C. C. *Endocrinol.*, **26**, 449, 1940.
37. HOFFMAN, J. "Female Endocrinology", p. 349, W. B. Saunders Co., Philadelphia, 1944.
38. KURZROK, R., and O'CONNELL, C. P. *Endocrinol.*, **23**, 476, 1938.
39. ROBSON, J. M. *Proc. Soc. Exper. Biol. & Med.*, **36**, 153, 1937.
40. GROLLMAN, A. "Essentials of Endocrinology", J. B. Lippincott Co., Philadelphia, 1941.
41. MCKEOWN, T., and ZUCKERMAN, S. *Proc. Roy. Soc. Med.*, **124**, 362, 1937.
42. BROOKSBY, J. B. *Proc. Soc. Exper. Biol. & Med.*, **38**, 235, 1938.
43. NATHANSON, I. T., and TOWNE, L. E. *Endocrinol.*, **25**, 754, 1939.
44. SALMON, U. J. *J. Clin. Endocrinol.*, **1**, 162, 1941.
45. GREENBLATT, R. B., MORTARA, F., and TORPIN, R. *Am. J. Obst. & Gynec.*, **44**, 658, 1942.
46. NATION, E. F., EDMONDSON, H. A., and HAMMACK, R. W. *Arch. Surg.*, **48**, 515, 1944.
47. SELYE, H. *Proc. Soc. Exper. Biol. & Med.*, **46**, 142, 1941.
48. SELYE, H. *Canad. M. J.*, **42**, 113, 1940.
49. SELYE, H. *Endocrinol.*, **32**, 116, 1943.
50. ENGLE, E. T. *Am. J. Physiol.*, **88**, 101, 1929.
51. WOLFE, J. M., and BROWN, A. D. *Endocrinol.*, **31**, 467, 1942.
52. MIN-CHUEH CHANG. *J. Endocrinol.*, **3**, 192, 1942.
53. GOLD, S. *Canad. M.A.J.*, **48**, 231, 1943.
54. CLAUBERG, C., and BREIPOHL, W. *Klin. Wchnschr.*, **14**, 119, 1935.
55. HOHLWEG, W. *Klin. Wchnschr.*, **13**, 92, 1934.
56. KLAFTEN, E. *Ztschr. f. Geburtsh. u. Gynäk.*, **115**, 64, 1937.
57. CHAMORRO, A. *Klin. Wchnschr.*, **16**, 196, 1937.
58. ESKIN, I. A. *Bull. Exper. Biol. & Med. U.S.S.R.*, 7-8, 68, 1944.
59. ABARBANEL, A. R. Ann. Meet. of the Society for the Study of Internal Secretions, Chicago, 1944.
60. ABARBANEL, A. R., and GOODFRIEND, M. J. *Am. J. Obst. & Gynec.*, **40**, 1037, 1940.
61. ZUCKERMAN, S. *Lancet*, **2**, 676, 1937.
62. GEIST, S. H., and SALMON, U. J. *J. Clin. Endocrinol.*, **1**, 154, 1941; *ibid.*, **1**, 162, 1941.
63. DANTCHAKOFF, V. *Comptes Rend. Soc. de Biol.*, **123**, 873, 1936.
64. TALBOT, H. B., BUTLER, A. M., and MACLACHLAN, E. A. *J. Clin. Invest.*, **22**, 583, 1943.
65. MCCULLAGH, E. P., JONES, R. *Cleveland Clin. Quart.*, **8**, 79, 1941.
66. LEWIS, L. A., and MCCULLAGH, E. P. *J. Clin. Endocrinol.*, **2**, 502, 1942.
67. MCCULLAGH, E. P., and LEWIS, L. A. *J. Clin. Endocrinol.*, **2**, 507, 1942.
68. ARMSTRONG, C. D. *J. Clin. Endocrinol.*, **4**, 23, 1944.
69. KORENCHEVSKY, V., and HALL, K. *J. of Endocrinol.*, **4**, 103, 1945.

70. THOMPSON, W. O. *J.A.M.A.*, **125**, 15, 1944.
71. WOLFE and CO-WORKERS quoted by ZONDEK, H. "The Diseases of the Endocrine Glands", Edward Arnold & Co., London, 1944.
72. MCEUEN, C. S., SELYE, H., and COLLIP, J. B. *Proc. Soc. Exper. Biol. & Med.*, **63**, 390, 1937.
73. THOMPSON, W. O. *J.A.M.A.*, **125**, 15, 1944.
74. ENGELHART, E., and SCHRANK, P. *Arch. f. Gynäk.*, **172**, 129, 1941.
75. DAVIDSON, C. A., and MOORE, H. D. *Proc. Soc. Exper. Biol. & Med.*, **35**, 281, 1935.
76. HALL, K., and KORENCHEVSKY, V. *J. Physiol.*, **91**, 365, 1938.
77. ARMSTRONG, C. D. *J. Clin. Endocrinol.*, **4**, 23, 1944.
78. REIFENSTEIN, E. C., JR., FORBES, A. P., ALBRIGHT, F., and DONALDSON, E., and CARROLL, E. *J. of Clin. Invest.*, **24**, 416, 1945.
79. BROSTER, L. R., CLIFFORD, ALLEN, VINES, H. W. C., PATTERSON, JOCELYN, GREENWOOD, ALAN W., MARRIAN, G. F., BUTLER, G. C. "Adrenal Cortex and Intersexuality", Chapman & Hall, London, 1938.
80. NATHANSON, J. T., TOWNE, L. E., and AUB, J. C. *Endocrinol.*, **24**, 335, 1939; *ibid.*, **28**, 851, 1941.
81. HAMBLIN, E. C. "Endocrinology of Women", Chas. C. Thomas, Springfield, Illinois, 1945.
82. ENG, H. *Klin. Wchnschr.*, **15**, 349, 1936.
83. ZONDEK, B., and EULER, H. *Skandinav. Anat. J. Physiol.*, **67**, 259, 1934.
84. D'AMOUR, F. E. *J. Clin. Endocrinol.*, **3**, 41, 1943.
85. DINGEMAUSE, E., LAQUER, E., and MUHLBOCK, O. *Nature*, **141**, 927, 1938.
86. BISKIND, MORTON, S. and L. R. *Endocrinol.*, **32**, 97, 1943.
87. HURXTHRAL, LEWIS, M. *J. Clin. Endocrinol.*, **3**, 551, 1943.
88. BISHOP, P. M. F., and FOLLEY, S. J. *Lancet*, **246**, 434, 1944.
89. WATKINSON, G., MCMENEMEY, W. H., and EVANS, G. *Lancet*, **252**, 631, 1947.
90. WYATT, J. *J. Obst. & Gynec. Brit. Emp.*, **52**, 174, 1945.
91. MORGAN, T. N. *Practitioner*, **154**, 149, 1945.
92. HERRMANN, J. B., and ADAIR, F. E. *J. Clin. Endocrinol.*, **6**, 769, 1946.
93. LISSER, H., and CURTIS, L. E. *J. Clin. Endocrinol.*, **3**, 389, 1943.
94. HIRST, J. C. *Am. J. Obst. & Gynec.*, **53**, 483, 1947.
95. FREED, S. C. *J.A.M.A.*, **127**, 377, 1945.
96. GREENBLATT, R. B., and KUPPERMAN, H. S. *J. Clin. Endocrinol.*, **6**, 675, 1946.
97. WERNER, A. A. *J.A.M.A.*, **127**, 705, 1945.
98. SIMONSON, E., KEARNS, W. M., and ENZER, N. *J. Clin. Endocrinol.*, **4**, 528, 1944.
99. FELDMAN, S., POLLOCK, J., and ABARBANEL, A. R. *Arch. Dermat. & Syph.*, **46**, 112, 1942.
100. SHELTON, E. K., and VARDEN, A. E. *J. Clin. Endocrinol.*, **6**, 812, 1946.
101. LISSER, H., and CURTIS, L. E. *J. Clin. Endocrinol.*, **5**, 363, 1945.
102. GLASS, S. J., and JOHNSON, B. H. *J. Clin. Endocrinol.*, **4**, 540, 1944.

103. MARQUEZ, A. L. *Semana Med.*, **1**, 1180, 1943.  
104. KUGELMASS, I. N. *J. Clin. Endocrinol.*, **6**, 823, 1946.  
105. LESSER, M. A. *J. Clin. Endocrinol.*, **6**, 549, 1946.  
106. MCGAVACH, T. H. *J. Clin. Endocrinol.*, **3**, 71, 1943.  
107. LESSER, M. A. *New England J. Med.*, **228**, 195, 1943.  
108. LEVINE, S. A., LIKOFF, W. B. *New England J. Med.*, **229**, 770, 1943.  
109. HAMM, L. *J. Clin. Endocrinol.*, **3**, 421, 1943.  
110. DOBES, W. L., JONES, J., and FRANKS, A. G. *J. Clin. Endocrinol.*, **5**, 412, 1945.  
111. SEVERINGHAUS, E. L., and SIKKEMA, S. *J. Clin. Endocrinol.*, **6**, 415, 1946.  
112. GLASS, S. J. *J. Clin. Endocrinol.*, **3**, 421, 1943.  
113. FREED, S. C. *J. Clin. Endocrinol.*, **6**, 571, 1946.  
114. SCHULTZ, F., and ANDERSON, C. E. *J. Clin. Endocrinol.*, **3**, 405, 1943.  
115. PRAGER, J. B. *Med. Rec.*, **152**, 261, 1940.  
116. DEAKINS, M. L., FRIEDGOOD, H. B., and FERREBEE, J. W. *J. Clin. Endocrinol.*, **4**, 376, 1944.  
117. REISS, M., and GOLLA, Y. M. L. *Endocrinol.*, **38**, 65, 1946.  
118. REIFENSTEIN, E. D., JR., and ALBRIGHT, F. *J. Clin. Invest.*, **26**, 24, 1947.  
119. WERNER, S. C., and WEST, R. *J. Clin. Invest.*, **22**, 335, 1943.  
120. ESCAMILLA, R. F., ROBERTS, F., and LISSER, H. *Clinics*, **1**, 170, 1942.  
121. KINSELL, L. W., HERTZ, S., and REIFENSTEIN, E. C. *J. Clin. Invest.*, **23**, 880, 1944.  
122. GREENBLATT, R. B. *J.A.M.A.*, **121**, 17, 1943.  
123. GEIST, S. H., and SALMON, U. J. *New York State J. Med.*, **41**, 222, 1941.  
124. GREENBLATT, R. B. *Am. J. Obst. & Gynec.*, **45**, 299, 1943.  
125. HIRST, J. C. *Am. J. Obst. & Gynec.*, **46**, 97, 1943.  
126. MILLER, J. R. *J.A.M.A.*, **125**, 207, 1944.  
127. SALMON, U. J., and GEIST, S. H. *J. Clin. Endocrinol.*, **3**, 235, 1943.  
128. RUBINSTEIN, H. S., SHAPIRO, H. D., and FREEMAN, W. *Am. J. Psychiat.*, **97**, 703, 1940.  
129. DUCKMAN, S., and TRURINO, T. R. *Am. J. Obst. & Gynec.*, **44**, 112, 1942.  
130. FARROW, J. L., and WOODWARD, H. O. *J.A.M.A.*, **118**, 339, 1942.  
131. FELS, E. J. *Clin. Endocrinol.*, **4**, 121, 1944.  
132. ABEL, S. *Am. J. Obst. & Gynec.*, **49**, 327, 1945.  
133. DAVIS, J. W. *Indust. Med.*, **11**, 422, 1942.  
134. MCSHAN, W. H., and MEYER, R. K. *J. Biol. Chem.*, **126**, 361, 1938.  
135. CHU, J. P., and YOU, S. S. *Endocrinol.*, **4**, 431, 1946.  
136. FEVOLD, H. L. *J. Biol. Chem.*, **128**, 83, 1939.



## THE PINEAL AND THYMUS

## THE PINEAL

THE human pineal gland is cone-shaped, reddish-grey in colour, and about 8 mm. long, 6 mm. wide and 4mm. thick. The average weight of the gland in normal conditions is from 0.160 to 0.167 gm. [1]. The gland lies in a depression between the two superior colliculi and the anterior corpora quadrigemina. The pineal stalk extends backwards to the roof of the third ventricle. The gland is separated from the corpus callosum by the tela choriodea.

Its secretory function, if any, is still obscure. The round or elongated epithelial cells within the reticular framework of vascular connective tissue show little evidence of secretory function. Extirpation, implantation and administration of pineal extracts have yielded contradictory results. Pinealectomy in the experimental animal has shown no changes in somatic or genital growth [2, 3]. Administration of pineal extract has resulted in gain in weight and size [4]. Other investigations have shown either no effect on growth [5] or inhibition of growth [6].

Some controversy exists as to the effects of pineal extract on gonadal function. A gonadal stimulating effect has been reported by Rowntree and others [7], and an oestrus-inducing effect by Saphir [8]. On the other hand, negative results are reported by Wade [9] and Tarkham [10]. Recently, an active fraction of the pineal gland has been prepared which delayed the onset of vaginal opening in the infant female mouse. Higher doses prolonged the period of inhibition [11].

The rare occurrence of pineal tumours associated with sexual precocity exclusively occurring in males (macrogenitosomia praecox) has led some observers to believe that precocious genital development is due to abnormal function of the pineal gland; on the other hand, there are reports of pineal tumours without sexual precocity and of such precocity in the absence of pineal tumour. The more likely explanation is that sexual changes are due to the pressure of the pineal tumour on the adjacent brain structure. The pressure of the tumour, particularly on the corpora quadrigemina, may result in loss of pupillary reaction to light and in impaired ocular mobility [12].

The pineal glands of 30 patients suffering from malignant tumours have recently been investigated by Kutscherenko [1] who found that the average weight of the gland (0.145 gram) was lower

than the figures known for the average weight of the gland under normal conditions. Histological examination of the gland in these patients showed a remarkably high proportion of calcareous spherulites, sometimes calcification of the blood-vessels, fuchsin staining of the pineal and nervous elements, and formation of cysts.

## THYMUS

The internal secretory function of the thymus is still in dispute. On histological examination the gland shows lympho-reticular tissue rather than cells with secretory functions.

The influence of thymus on growth is also uncertain. Recent reports show that thymectomy in rats is compatible with normal growth [13, 14, 15]. Growth-stimulating effect following the administration of thymus extract was noted by Rowntree and others [16]. However, negative results are reported by Burrill [17] and Segaloff [18]. The growth-promoting effect of the thymus gland is attributed to its active substance, glutathione [19, 20, 21, 22, 23]. No effect on growth, however, was obtained with glutathione by Segaloff and Nelson [18].

In experiments with mice of a highly leukaemic strain (AK) it has been shown that the removal of the thymus has the effect of prolonging life and of decreasing the incidence of leukaemia from 80·8 per cent to 9·9 per cent [47].

### Myasthenia Gravis

In patients suffering from myasthenia gravis, synthesis of acetylcholine necessary for the transmission of nervous impulses is inhibited. It has been demonstrated that the thymus is the responsible inhibiting factor [40]. Marked improvement of myasthenia gravis following thymectomy is reported by some authors [41, 42, 43], but only partial improvement [44, 45] or negative results [46] by others. Examination of the removed glands shows an increase in the number of lymphocytes and loss of the normal sharp differentiation between the cortex and medulla [42].

On the basis of their operative findings Clagett and Eaton [53] believe that the results to date warrant thymic exploration in all cases of myasthenia gravis in which the disease is severe or progressive and in which the condition will permit surgery.

## RELATION OF THE THYMUS TO OTHER ENDOCRINE GLANDS

**Anterior Pituitary Gland**

It seems that anterior pituitary function influences the thymus gland. Thymus involution has been observed in animals [25, 24], after hypophysectomy; while acromegaly may be associated with thymus hyperplasia [26].

Administration of growth hormone has been found to produce a marked increase in the size of the thymus [24], and hyperthyroidism is associated with a striking lack of thymus involution. Such involution, however, is stimulated by the gonadotrophic [27], the corticotrophic [28], and thyrotrophic [29] hormones; and, to complete the picture, thymus extract administered to the rat results in pituitary enlargement with an increase in the number of eosinophils [30].

Although a thymotrophic hormone has not yet been isolated, it appears reasonable to assume that one is produced by the pituitary, though its seat of production is still a matter of speculation.

**Gonadal System**

Thymectomy does not apparently influence the size of the gonads or the onset of puberty [31]. Administration of thymus extract, however, causes growth of the genitalia and sexual precocity in the offspring of rats [16].

The gonadal system appears to inhibit the thymus, sexual development, or normal or precocious puberty, causing its involution. Status thymicolymphaticus is usually associated with gonadal hypoplasia. Castration in the rat results in thymus hypertrophy [32, 33, 34]. Oestrogen causes involution of the thymus [35], and prevents thymus hyperplasia following ovariectomy in the adult female rat. The non-oestrogenic sterols do not cause thymic involution.

**Thyroid**

Graves's disease is often associated with hyperplasia of the thymus, just as myxoedema often is with its atrophy. Experimental evidence suggests that the relation of the thymus to the thyroid gland is one of antagonism [36, 37]. The thymus hyperplasia in Graves's disease is explained by Bomskov as a compensatory reaction.

**Adrenals**

The relations of the thymus to the adrenal glands is one of antagonism. Persistent thymus hyperplasia is met with in adrenal cortical



deficiency. It seems that the medulla takes part in the antagonism to the thymus, for congenital hypoplasia of the medulla is consistently associated with hyperplasia of the thymus and the whole lymphatic system.

Adrenal cortical extract has a beneficial effect on status thymico-lymphaticus in children [38], and the corticotrophic hormone causes a reduction in size and complete regression of the thymus [28]. According to Selye, the "alarm reaction," which is characterized by a sudden increase in the secretion of the cortical hormone, is followed by involution of the thymus (see Chapters I, V).

The brain of animals injected with the adrenotrophic hormone synthesized more acetylcholine than that of non-injected animals. Most steroid hormones decreased the amount of acetylcholine synthesized, but oestrogenic hormones and  $\Delta^5$ -pregnenolone increased the synthesis [48]. Thyroxine increased the acetylcholine synthesis [49], as did epinephrine [50]. The effect of epinephrine in increasing the amount of acetylcholine synthesized may explain why epinephrine increases the effect of acetylcholine in the central nervous system and improves transmission from nerve to the muscle [51].

Vitamins may modify the amount of acetylcholine synthesized. Vitamin E, even in very low concentrations, vitamin C, and most members of the B group increase the amount of acetylcholine synthesized. Vitamin A and K in all the concentrations used, thiamine chloride and vitamin D in higher concentrations decrease the synthesis [52].

### Pancreas

There is some evidence that the relation to the pancreas is one of antagonism, since irradiation of the thymus in dogs leads to hypertrophy and hyperplasia of the islet system [39].

## BIBLIOGRAPHY

### THE PINEAL

1. KUTSCHERENKO, B. P. *Problems of Endocrinology U.S.S.R.*, **1**, 131, 1941.
2. D'AMOUR, M. C. and F. E. *Proc. Soc. Exper. Biol. & Med.*, **37**, 244, 1937.
3. ANDERSEN, D. H., and WOLF, A. *J. Physiol.*, **81**, 49, 1934.
4. ADAIR, J., and CHIDESTER, F. E. *Endocrinol.*, **12**, 791, 1928.
5. KOZELKA, A. W. *Proc. Soc. Exper. Biol. & Med.*, **30**, 842, 1933.
6. ROWNTREE, L. G., CLARK, J. H., STEINBERG, A., and HANSON, A. M. *J.A.M.A.*, **106**, 370, 1936.

7. ROWNTREE, L. G., CLARK, J. H., STEINBERG, A., HANSON, A. M., EINHORN, N. J., and SHANNON, W. A. *Ann. Int. Med.*, **9**, 359, 1936.
8. SAPHIR, W. *Endocrinol.*, **18**, 625, 1934.
9. WADE, N. J. *Endocrinol.*, **21**, 681, 1937.
10. TARKHAM, A. A. *Endocrinol.*, **18**, 234, 1937.
11. FISCHER, E. *Endocrinol.*, **33**, 116, 1943.
12. GLADSTONE, R. J., and WAKELEY, C. P. G. "The Pineal Gland", Baillière, Tindall, & Cox, London, 1940.

#### THE THYMUS

13. HASIMOTO, E. J., and FREUDENBERGER, C. B. *J.A.M.A.*, **112**, 1680, 1939.
14. CHIODO, H. *Compt. Rend. Soc. de Biol.*, **130**, 298, 1939.
15. SEGALOFF, A., and NELSON, W. O. *Am. J. Physiol.*, **130**, 671, 1940.
16. ROWNTREE, L. G., CLARK, J. H., HANSON, A. M., and STEINBERG, A. *J.A.M.A.*, **103**, 1425, 1934.
17. BURRILL, N. W., and IVY, A. C. *Endocrinol.*, **28**, 94, 1941.
18. SEGALOFF, A., and NELSON, W. O. *Endocrinol.*, **29**, 483, 1941.
19. GUDERNATCH, J. F. *Med. Rec.*, **146**, 101, 1937.
20. HOFFMANN, O., and GUDERNATCH, J. F. *Am. J. Physiol.*, **97**, 527, 1931; *Proc. Soc. Exper. Biol. & Med.*, **28**, 731, 1931; *Am. J. Physiol.*, **113**, 67, 1930.
21. ROWNTREE, L. G., CLARK, J. H., STEINBERG, A., EINHORN, N. H. *N.Y. State Med. J.*, **36**, 1277, 1936.
22. ROWNTREE, L. G., STEINBERG, A., EINHORN, M. H., and SCHAFFER, N. K. *Endocrinol.*, **23**, 584, 1938.
23. SCHAFFER, N. K., ZIEGLER, W. M., and ROWNTREE, L. G. *Endocrinol.*, **23**, 593, 1938.
24. UYLDERT, I. E., and FREUD, J. *Acta. brev. Neerland*, **8**, 188, 1938.
25. HOUSSAY, B. A., and LASCANO-GONZALEZ, J. M. *Compt. Rend. Soc. de Biol.*, **117**, 463, 1934.
26. SLOAN, H. E. *J. Surg.*, **13**, 154, 1945.
27. EVANS, H. M., and SIMPSON, M. E. *Anat. Rec.*, **60**, 423, 1934.
28. EVANS, H. M., MOON, H. D., SIMPSON, M. E., and LYONS, W. R. *Proc. Soc. Exper. Biol. & Med.*, **38**, 419, 1938.
29. WATRIN, J., and FLORENTIN, P. *Compt. Rend. Soc. de Biol.*, **110**, 1161, 1932.
30. CLARK, J. H., STEINBERG, A., and ROWNTREE, L. G. *Proc. Soc. Exper. Biol. & Med.*, **35**, 239, 1936.
31. CHIODI, H. *Compt. Rend. Soc. de Biol.*, **130**, 289, 1939.
32. MOORE, C. R. *Am. J. Anat.*, **59**, 63, 1936.
33. CHIODI, H. *Compt. Rend. Soc. de Biol.*, **130**, 457, 1939.
34. LAUSON, H., HELLER, C. G., and SEVERINGHAUS, E. L. *Endocrinol.*, **21**, 735, 1937.
35. SELYE, H., HARLOW, C. M., and COLLIP, J. *Endocrinol.*, **18**, 81, 1936.

6. CROTTI, A. "Diseases of the Thyroid, Parathyroids and Thymus", pp. 978-1040, Lea & Febiger, Philadelphia, 1938.
7. BOMSKOV, C., and SPIEGEL, R. *Endocrinol.*, **23**, 225, 1941.
8. KEMP, W. M. *Brit. Med. J.*, **1**, 1194, 1937.
9. BENTIVOGLIO, G. C., and FUMI, C. *Sperimentale Arch. di Biol.*, **9**, 219, 1937.
10. TORDA, CLARA, and WOLFF, H. E. *Proc. Soc. Exper. Biol. & Med.*, **57**, 69, 1944.
11. NELLEN, MAURICE. *Brit. Med. J.*, **2**, 778, 1943.
12. SLOAN, H. E., JR. *Surg.*, **13**, 154, 1943.
13. POER, D. H. *Ann. Surg.*, **115**, 586, 1942.
14. VIETS, H. R. *J.A.M.A.*, **127**, 1089, 1945.
15. BLALOCK, A. *Thorac. Surg.*, **13**, 316, 1944.
16. TURNBULL, F. *Arch. Neurol. Psychiat.*, **48**, 938, 1942.
17. FURTH, J. *J. Gerontol.*, **1**, 46, 1946.
18. TORDA, C., and WOLFF, H. G. *Proc. Soc. Exper. Biol. & Med.*, **57**, 327, 1944.
19. *Ibid.*, **59**, 181, 1945.
20. *Ibid.*, **56**, 86, 1944.
21. BURN, J. H. *Physiol. Rev.*, **25**, 377, 1945.
22. TORDA, C., and WOLFF, H. C. *Science*, **103**, 645, 1946.
23. CLAGETT, O. T., and EATON, L. M. *J. of Thoracic Surg.*, **16**, 62, 1947.



ENVIRONMENTAL INFLUENCES  
ON GLANDULAR FUNCTION

THE function of the endocrine glands is influenced by light, temperature, moisture and atmospheric pressure.

Light controls hypophyseal activity, particularly its gonadotrophic function [1], and constantly applied to the female rat, it causes constant follicular development without ovulation [2].

High environmental temperature causes decreased thyroid function, with a reduction in the height of the epithelial cells and an accumulation of a dense homogeneous colloid in the follicles. The thyroid glands of animals living in cold climates, or exposed to cold, show a high epithelium and increased function [1, 2].

The adrenal gland increases in size upon prolonged exposure to cold temperature [3, 4]. The lipid content of the adrenal cortex decreases in winter and increases in summer, lipid loss denoting greater activity in the winter season [5].

Simultaneous exposure to cold and light produces constant oestrus in the female rat, and also stimulates the adrenal and thyroid glands. All of these reactions, with the exception of the thyroid stimulation by cold, may be produced in animals after pituitary stalk section, a fact denoting that environmental stimuli may be transmitted to the anterior pituitary by routes other than the pituitary stalk [2].

It is suggested that parathyroid function increases during the winter. Chorio-allantois grafts in the chicken embryo produced typical chondrodystrophy almost exclusively during the colder months [6, 7].

Atmospheric pressure is another climatic factor of importance. Low atmospheric pressure produces adrenal hypertrophy [8]. Rats long exposed to very low pressure develop a syndrome closely resembling cortico-adrenal insufficiency, and it is suggested that mountain sickness in man may be due to failure of the adrenal cortex to respond to low pressure by increased activity, or to adrenal cortical exhaustion [9]. In addition to an increase in adrenal weight, decreases in the weights of the testes, seminal vesicles, ventral prostate, thymus and possibly thyroid have been noted in rats discontinuously exposed to low atmospheric pressure. Pituitary gland weights were not affected.

The decrease in testicular weight is mainly due to degeneration of the germinal epithelium with only slight atrophy of the interstitial

issue. This is reflected in increased anterior pituitary lobe gonadotrophic activity, with an increase in the number of basophil cells and the appearance of castration-like cells. Decrease of thyroid activity is not marked and there is no reduction in the pituitary content of thyrotrophic hormone [10].

## BIBLIOGRAPHY

1. VOITKEVITCH, A. A. *Bull. Exper. Biol. & Med. U.S.S.R.*, XV, 9, 16, 1943.
2. DEMPSEY, E. W., and SEARLES, H., Dept. of Anatomy, Harvard Medical School, Boston, Mass. *Endocrinol.*, 2, 32, 1943.
3. EMERY, F. E. and L. M., and SCHWABE, E. L. *Growth*, 4, 17, 1940.
4. SELYE, H. *Endocrinol.*, 21, 169, 1937.
5. BERNSTEIN, J. G. *Endocrinol.*, 28, 985, 1937.
6. STUDITSKY, A. N. Unpublished communication.
7. STUDITSKY, A. N. *Comptes Rend. de l'Acad. des Sci. de l'U.S.S.R.*, 18, No. 9.
8. ARMSTRONG, H. G., and HEIM, G. W. *An. Med.*, 9, 92, 1938.
9. SANDSTROEM, E. S., and MICHAELS, J. "The Adrenal Cortex in Adaptation to Altitude Climate and Cancer", Univ. of California Press, Berkeley and Los Angeles, 1942.
10. GORDON, A. S., TORNETTA, F. G., and CHARIPPER, H. A. *Proc. Soc. Exper. Biol. & Med.*, 53, 6, 1943.

## PSYCHO-SOMATIC ENDOCRINOLOGY

THE relation between the endocrine system, the autonomous nervous system, cerebral function, the bodily organs and tissues, and environmental factors, represents a complex network of mutual excitation and inhibition (see Fig. 14). The constant interaction of strong antagonistic and stimulating forces maintains the organism in equilibrium. Abnormal changes, active or passive, affecting any part concerned in the maintenance of this balance, may easily create a vicious circle with progressive deterioration. On the other hand, successful therapeutic

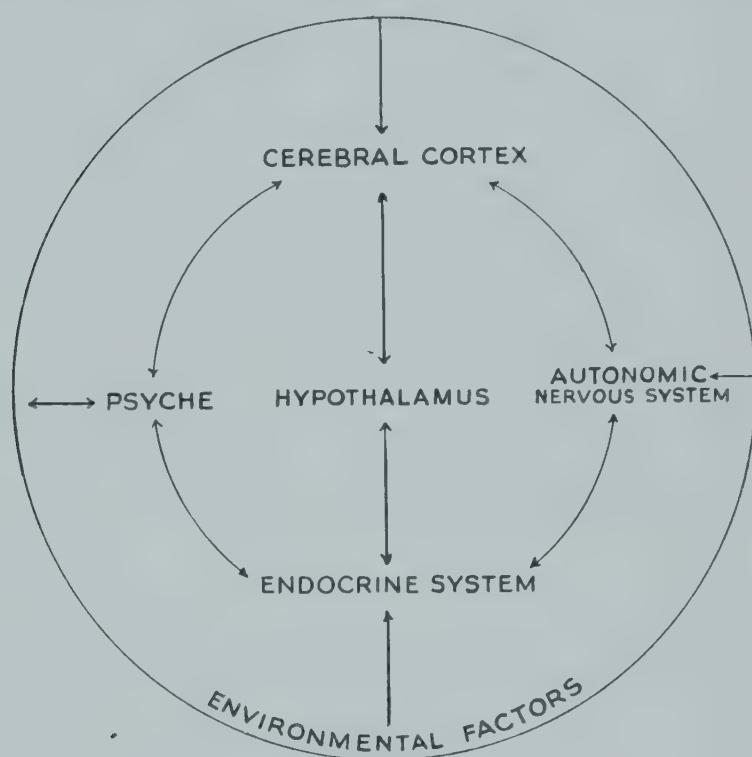


FIG. 14.—CEREBRO-GLANDULAR INTERACTION

intervention or spontaneous regression to normal at any one point, may interrupt the vicious circle and re-establish normal function.

Pathological changes of glandular function may easily cause mental disturbances, ranging from slight emotional upsets to severe psychoses. The glandular disorder may act directly on cerebral function (as in hyperthyroidism, hypothyroidism, hypoglycaemia, Addison's disease, etc.) or, indirectly, by causing some physical impediment (obesity, impotence, etc.) which leads to psychosis by way of emotional strain and frustration.

On the other hand, psychic influences are powerful factors in



determining endocrine function and in creating severe glandular disturbances. Some familiar examples are Cannon's emergency reaction; the precipitation of Graves's disease with concomitant adrenal hypofunction and amenorrhoea by psychic trauma; psychogenic hyperglycaemia; functional impotence, and anorexia nervosa.

In some cases a common aetiological factor leads simultaneously to the endocrine and mental disturbances—e.g. a hypothalamic lesion, intracranial pressure, encephalitis, etc.

## THE EFFECT OF HORMONAL CHANGES ON THE PSYCHE

The endocrine changes which occur at various phases in human life are often accompanied by mild or severe emotional upsets, irrespective of whether the transient or permanent alterations in the glandular secretions are progressive or involutional in character.

### PUBERTY

The psychological disturbances of puberty are often manifestations of constitutional inadequacy, due to such causes as heredity, consanguinity, alcoholism in the parents, unfavourable environmental conditions in the formative years, etc. The conditions themselves range from simple neuroses to frank psychoses.

The physical manifestations which occur during puberty suggest that the changes in the endocrine system may be partly responsible for the psychological disturbances. The appearance of erotic and heterosexual tendencies in either sex is probably due to the onset of gonadal function. The participation of the ovarian and testicular hormones in the endocrine concert often causes difficulties in readjustment of the glandular system. Thus emotional disturbances like moodiness, shyness, stubbornness and irresponsibility accompanied by nervous irritability, insomnia, restlessness and vaso-motor instability with blushing, pallor, sweats and tachycardia are not uncommon. Acne frequently occurs at puberty and is cyclic in girls while it persists in boys until late adolescence. The underlying cause is probably a shift in the oestrogen-androgen ratio towards a temporary unduly increased androgenic level and is in both sexes alleviated by oestrogenic therapy.

### MENSTRUAL CYCLE

There is close relationship between the emotional changes and the hormonal balance during the menstrual cycle; and the following conclusions may be drawn from the literature on the subject.

### **Follicular Phase**

With the production of increasing amounts of oestrogen, the emotional state is dominated by an active sexual drive connected with the feeling of well-being and alertness. The sexual drive has a marked heterosexual character. If the sexual desire is gratified, its psychic expression becomes less marked; if not, restlessness and irritability develop and, in some women, a state of anxiety.

### **Ovulatory Phase**

This phase is characterised by an increase in sexual desire, and greater readiness for coitus. With ovulation comes a temporary release of tension and a sense of well-being. This is distinct from the pre-ovulatory phase by its marked calmness and change of attitude. The sexual impulse takes a passive direction with orientation towards the woman's own body as the object of libido. The genital organs become more sensitive, and there is increased receptivity for the sexual partner. At about this phase the woman reaches the highest level of psycho-sexual integration of which she is capable [2].

### **Luteal Phase**

During this phase, the body temperature and basal metabolic rate are raised. The heterosexual tendency is in the background while the passive receptive tendency is dominant. After the post-ovulatory phase of relaxation, the luteal phase may sometimes show a conflict between the active heterosexual tendency of the follicular phase and the passive attitude of the early luteal phase. Benedek and Rubinstein [2] observed, in this phase, the development of a psychological state similar to the one considered responsible for depression. An increased narcissism with regression to an infantile dependance and receptivity has been noted.

### **Premenstrual Phase**

This phase is characterized by the reappearance of oestrogen, with a heterosexual direction of the psychological attitude. The change in sexual attitude corresponds to the oestrogen-progestogen balance. Benedek and Rubinstein divide this phase into the early and the late premenstrual phase.

#### **Early Premenstrual Phase**

This is characterized by usually low hormone production, progestogen continuing to decrease while oestrogen production remains low.

Hence, the psychological tendencies are characteristic of the deficiency of both the hormones and the conflicts are not so sharply defined. The authors have observed that even highly-sexed women appear somewhat to regress emotionally during this phase of the cycle.

### Late Premenstrual Phase

According to Benedek and Rubinstein the late premenstrual phase is characterized by variation in the hormonal balance with corresponding variation in the psychological state. The chief variations, as seen in different women, are grouped as follows:

1. Decrease of progesterone (*a*) with no increase of oestrogen, or (*b*) with slight increase of oestrogen.
2. Increase of oestrogen while progesterone is maintained at a low level.
3. Decrease of both hormones.

#### 1. *Decrease of progesterone with slight increase of oestrogen*

The emotional state is determined by two factors:

- (*a*) The eliminative tendency corresponding with the decrease of progesterone, and
- (*b*) the heterosexual tendency, corresponding with oestrogen production.

The interplay of these factors is characterized by emotional tension, irritability and impatience. An active sexual urge, unlike the receptive love-desiring tendency in the luteal phase, is observed in this state.

#### 2. *Increase of oestrogen while progesterone is maintained at a low level*

The hormonal balance is similar to that of the pre-ovulatory phase. The psychological state is characterized by impatience even more marked and sexual demands more urgent than those previously described. Anxiety, increased to the point of desperation, and aggression expressed in violent rage or turned inward suicidally, have been observed in this hormonal state [2].

#### 3. *Decrease of both hormones*

This is often characterized by depression, and in nearly all women by some decline in intellectual activity.



### Menstrual Phase

The most outstanding reaction at the beginning of this phase is depression. Chadwick has described the emotional state as one of tearfulness, suspicion and inclination to self-pity or self-reproach, in addition to a general feeling of low spirits and irritability with the accompaniment of frightening dreams. After the onset of the menstrual flow, the excitability decreases, the tense fearful mood relaxes, and the hostile or depressed emotional state is usually relieved [2].

### PUERPERAL PSYCHOSES

The potentially psychotic personality is prone to develop puerperal psychosis. The prognosis is good in the delirious and in the manic-depressive groups, but far less hopeful when the reaction is of the schizophrenic type. Apparently 20 per cent or less of the patients recover spontaneously. Insulin shock therapy increases the recovery rate. It is worth recalling that the patients who develop puerperal psychoses are often those who suffer from premenstrual tension. One patient who showed only partial recovery after insulin shock, responded well to progestogen treatment [3].

### MATERNAL FEELING

Levi [4], in interviews with 72 women nearly all of them mothers of children under treatment for behaviour disorders, found a relationship between the habitual number of days of menstrual flow and the strength of maternal feeling. Thus, a significantly higher proportion of strongly maternal women belonged to the 6-8 days than to the 2-4 days group. .

### CLIMACTERIC

Diminution in ovarian function with increased activity of the anterior pituitary basophil cells leads to a disturbance in the whole endocrine system accompanied by over-responsiveness of the sympathetic nervous system, associated with the characteristic hot flushes and psychic disturbances. These become manifest as irritability, mysticism and religious excitation, eroticism of varying degrees, jealousy, suspicion and hypochondria. In some cases the symptoms are intensified by fears, doubts, phobias and compulsions [5].

## THE ENDOCRINE SYSTEM IN MENTAL DISORDERS

Mental fatigue, insomnia, restlessness and abnormal sensory reactions are often associated with adrenal cortical deficiency. In cases of involutional melancholia, due to pituitary and adrenal hypofunction, treatment with corticotrophic hormones results in improvement [7, 8, 9]. Patients successfully treated by shock show an increased output of urinary 17-ketosteroids [10].

### Epilepsy

The formerly practised unilateral adrenalectomy with resection of the contralateral gland produced striking benefit in a fairly large number of epileptics. Goldzieher [6] found that epileptiform convulsions and petit mal are often associated with salt and water retention, and can in some degree be prevented by injections of desoxycorticosterone acetate [25].

Desoxycorticosterone acetate apparently has a specific effect on salt and water metabolism, distinct from that of cortical extract. It causes accumulation of sodium and chloride and elicits increased elimination of water, thus protecting against "water intoxication". Cortical extract increases the potassium content of the cells, simultaneously removing sodium and increasing the amount of water in the extracellular spaces, i.e. that part of the tissue-water which is apparently responsible for the cerebral manifestations.

### Adrenogenital Syndrome

Adrenal cortical hypertrophy in the female causes the so-called "adrenogenital syndrome", accompanied by manic-depressive psychosis. In these cases, unilateral adrenalectomy results in physical and mental improvement [11, 12, 13].

Baird [14] gave adrenalectomized cats whole citrated blood obtained from manic-depressive patients during an acute crisis. The average survival time of these cats was about 5 times as long as that of adrenalectomized controls, which were characteristically weak, inactive and semi-agonal a few days after the removal of the adrenals. Two of the treated cats showed exceptional strength and activity, being capable of running without fatigue and offering powerful resistance during the injection. Moreover, one of them was highly excitable, easily enraged, wild and ferocious, requiring to be held for the injections by the combined strength of three men. Adrenalectomized rats that received manic blood also exhibited unusual strength

and activity, voracious appetite, and remarkable excitability and resistance during the injections.

#### THE THYROID IN RELATION TO MENTAL DISEASES

##### **Hyperthyroidism**

The effects of excess thyroid hormone on the nervous system are well known. The subject is usually emotionally unstable and irritable, and cerebration may be grossly disturbed, showing a tendency to the manic type of psychosis. Hyperfunction of the thyroid gland is usually connected with disturbances of gonadal and adrenal cortical function.

##### **Myxoedema**

Myxoedema is usually accompanied by slowness and slackness of mental reactions, lack of emotional response, and sometimes by psychoses of depressive or manic character with outbreaks of rage or fury. Other characteristic features are hallucinations of smell and less often of sight and hearing, delusion of persecution and a clouding of consciousness. Zondek and Wolfsohn [15] describe a case of a woman, 23 years old, who for 18 months suffered from myxoedema associated with schizophrenia. Mental recovery set in a few days after the beginning of thyroid treatment and was complete in a fortnight. Thyroid administration resulted in a large increase in urine output and increase of sodium and chloride excretion, although fluid intake was restricted. It is suggested that the improvement in the mental condition was due to dehydration of the brain by the mobilization of water from the extracellular cerebral tissues.

Electroencephalographic studies in myxoedema show a low cortical alpha rate which returns to normal under thyroid medication. It is suggested that the alpha rate can thus be a fair index of the state of metabolism [16].

#### HYPOGLYCAEMIA

The disorders in the endocrine system which may lead to hypoglycaemia are manifold and include, for example, hypopituitarism, hypothyroidism, adrenal cortical deficiency, liver dysfunction, and islet adenoma of the pancreas.

Among the varied neurological manifestations of hypoglycaemia may be mentioned troublesome hunger, weakness, palpitations, nervousness, anxiety, irritability, tremor, sweating, headache, inability to concentrate, fatiguability, insomnia, vertigo or dyspnoea, with, in the more severe cases, periods of actual unconsciousness and



transient haemiplegia. The most characteristic pathological feature of the condition, however, is the widespread degeneration of the nerve cells of the cerebral cortex, the basal ganglia and the cerebellum, seen in particular and most constantly in the cells of the caudate nucleus and putamen. Large parts of the cerebral cortex undergo necrosis, often of a pseudolaminar distribution, with severe degeneration of neurones. The mechanism responsible for the cell damage is a reduced oxygen utilization by the neurones, glucose being the main, and probably the only, substance which the brain can use for this oxidative process [17, 18].

#### ELECTROENCEPHALOGRAM

Variations in the electroencephalogram due to endocrine changes afford further evidence of the hormonal control of cerebral activity.

#### **Menstruation**

The onset of menses slows down the electrical activity of the cortex [19].

#### **Pregnancy**

Changes in the electroencephalogram during pregnancy reflect a decreased electrical activity of the cortex. A more high-voltage fast activity is seen in the presence of pregnancy toxæmias [20].

#### **Thyroid**

Myxoedema is associated with a low cortical alpha rate in the electroencephalogram, which returns to normal under thyroid treatment [16].

#### **Parathyroid**

Hyperventilation at the rate of 30 respirations in 45 seconds, which would not in the normal man produce noticeable modifications in the electroencephalogram, resulted in the following changes in patients suffering from post-surgical or spontaneous parathyroid insufficiency.

1. A marked tendency of the alpha rhythm to diminish or disappear in the occipital, parietal and, to lesser degree, in the frontal records.

2. An increased constancy and amplitude of the beta rhythm; it may be uniform, paroxysmal, or appear as a modulation of the alpha rhythm [21].

### **Suprarenal Glands**

A high proportion of patients with Addison's disease show an electroencephalogram with abnormalities in the resting pattern, and increased sensitivity to voluntary hyperventilation [22].

## **THE INFLUENCE OF MENTAL DISTURBANCES ON ENDOCRINE FUNCTION**

### **Psychic Trauma**

Absence of bleeding frequently occurs following emotional strain. Before the outbreak of war, many refugees had periods of amenorrhoea on their arrival in Britain. During the bombing of London there was a common history of sudden interruption in the cycle, usually associated with amenorrhoea.

Whiteacre [23] reports a high incidence of amenorrhoea among the women internees at the Santa Thomas Camp, Manila, in 1942. Of 1,042 women of menstrual age, 125 suffered from amenorrhoea. The menses stopped abruptly after the first bombing or soon after internment, and before food deficiency could have any effect.

### **Dementia Praecox**

The tests in over half of 90 schizophrenic patients investigated by Hemphill and others [24] showed, on testicular biopsy, atrophy of the seminiferous tubules with hyalinization of the basement membrane, cessation of spermatogenesis and progressive degeneration of epithelial elements with eventual destruction of the tubules. Of 25 control patients suffering from other forms of mental disorder, only three showed similar changes. These changes were also very rare in schizophrenics of the paranoid type; but they were found in the most advanced form not only in the chronic, catatonic and deteriorated cases, but also in early cases. Severe testicular atrophy was found in one boy of 15 who had been ill for only a few weeks. The excretion rates of 17-ketosteroids were normal. All cases showed decreased liver function, and Hemphill suggests that decreased testicular function may be due to failure of the liver to inactivate oestrogens, with a consequently high oestrogenic level inhibiting spermatogenesis.

### **Pseudocyesis**

The aetiology of pseudocyesis has been ascribed to a desire for pregnancy and a fear of it. The objective signs are not readily explained. A more satisfactory explanation of the underlying cause is however

provided by the noted significant increase in the excretion of gonadotrophins and oestrogens. The increased hormonal output is attributed to the influence of the psyche on the endocrine system. Moreover, when the patient is told that she is not pregnant the physical signs disappear and the hormonal titres return to normal [1].

## BIBLIOGRAPHY

1. STEINBERG, A., PASTOR, N., WINHELD, E. B., SEGAL, H. S., SCHECKTER, F. R., and COLTON, N. H. *Psychosom. Med.*, **8**, 176, 1946.
2. BENEDEK, THERESA, and RUBINSTEIN, B. "The Sexual Cycle in Women", p. 132, *Psychosom. Med. Monogr. National Research Council*, Washington, D.C., Vol. III, 1943.
3. BLUMBERG, A., and BILLIG, O. *Psychiat. Quart.*, **16**, 454, 1942.
4. LEVI, D. M. *Psycho-Somatic Med.*, **4**, 223, 1940.
5. QUARANTA, A. P. *An. Catedra de Clin. Ginec.*, **2**, 298, 1943.
6. GOLDZIEHER, M. A. "The Adrenal Glands in Health and Disease", F. A. Davis Co., Philadelphia, 1944.
7. HEMPHILL, R. E., and REISS, M. *J. Ment. Sc.*, **88**, 559, 1942.
8. MOORE, T. V. *Psychiat. Quart.*, **16**, 765, 1942.
9. HEMPHILL, R. E., and REISS, M. *Brit. Med. J.*, 5362, 211, 1944.
10. HEMPHILL, R. E., MCLEOD, L. D., and REISS, M. *J. Ment. Sc.*, **88**, 554, 1942.
11. ALLEN, C., and BROSTER, L. R. *Brit. Med. J.*, 4402, 696, 1945.
12. GREENE, R., PATTERSON, A. S., and LAWRIE PILE, G. C. *Brit. Med. J.*, 4402, 698, 1945.
13. ALLEN, C., and BROSTER, L. R. *Brit. Med. J.*, **1**, 696, 1945.
14. BAIRD, P. C., JR. *J. Nerve and Ment. Dis.*, **99**, 359, 1944.
15. ZONDEK, H., and WOLFSOHN, G. *Lancet*, **247**, 438, 1944.
16. ROSS, D. A., and SCHWAB, H. S. *Endocrinol.*, **28**, 75, 1939.
17. ROMANO and COON, *Psycho-Somatic Med.*, **4**, 283, 1942.
18. LAWRENCE, R. D., MEYER, A., and NEVIN, S. *Quart. J. Med.*, **11**, 181, 1942.
19. DUSSER DE BARENNE, D., and GIBBS, F. A. *Am. J. Obst. & Gynec.*, **44**, 687, 1942.
20. GIBBS, F. A., and REID, D. E. *Am. J. Obst. & Gynec.*, **44**, 672, 1944.
21. ODORIZ, J. B., DE CASTILLO, E. B., MANFREDI, J. F., and DE LA BALZE, F. A. *J. Clin. Endocrinol.*, **4**, 493, 1944.
22. HOFMAN, W. C., LEWIS, R. A., and THORN, G. W. *Bull. Johns Hopkins Hosp.*, **70**, 335, 1942.
23. WHITEACRE, F. E., and BARRERA, B. *J.A.M.A.*, **124**, 399, 1944.
24. HAMPHILL, R. E., REISS, M., and TAYLOR, J. *Ment. Sc.*, **90**, 681, 1944.
25. MCQUARRIE, J., ANDERSON, J. A., and ZIEGLER, M. R. *J. Clin. Endocrinol.*, **2**, 406, 1942.



NON-HORMONAL SUBSTANCES FOR THE  
TREATMENT OF ENDOCRINE DISORDERS

## THIOURACIL

INHIBITION of thyroid activity by means of thiouracil may, in many cases, take the place of partial thyroidectomy which, until recently, was the treatment of choice for thyrotoxicosis.

Hypothyroidism and enlargement of the gland were first induced in the rat with sulphaguanidine [1], allyl thiourea [2], and later with other sulphonamide derivatives [3, 4]. Clinical trials with thiourea and thiouracil in thyrotoxicosis were first reported in 1943 [5-7].

Thiouracil and thiourea are similar in action. Thiouracil, however, has the advantage of not producing the unpleasant smell in the breath, the conjunctivitis and the vomiting which frequently followed thiourea treatment [7].

**Mode of Action**

Thiouracil inhibits the conversion of iodine to thyroxin and diiodo-tyrosine [7, 8, 9], and its administration thus brings about complete depletion of iodine in the thyroid colloid [7, 10, 11]. Owing to the lack of thyroxin, there occurs increased anterior pituitary activity which causes thyroid hyperplasia, [7, 8, 12, 13].

**Anterior Pituitary**

The fact that thiouracil treatment does not produce thyroid hyperplasia in the hypophysectomized animal suggests that it acts through the anterior pituitary [8, 7, 10]. Sulphaguanidine causes a decrease in the number of eosinophils and enlargement with vacuolization of the basophil cells. Administration of thyroxin prevents these changes [3]. Similar changes occur after thyroidectomy and are reported with thiouracil [39] (see Fig. 15). Administered to the rat, it produces definite slowing of the rate of growth and tends to inhibit the action of growth hormone. The pituitary, gonads and adrenals are smaller in the treated animals. The adrenal cortex of rats fed with thiouracil undergoes involution which may reduce it to less than half its size if treatment is continued for 3 or 4 months. All three zones of the cortex are involved in this involution [34], which is similar to that which follows thyroidectomy.

Thiouracil augments the goitrogenic effect of the thyrotrophic hormone [14]. The changes in the gonads and adrenals are probably due to the lack of thyroxin. The effect of thiouracil in producing

increased activity of the basophil cells causes a decrease in the eosinophils which presumably produce the luteinizing and corticotrophic hormone. Since the lactogenic hormone is probably also secreted by the eosinophilic cells [40, 41] the administration of 0.1 per cent thiouracil in the feed for 24 days to young female rats has been found to reduce the lactogenic hormone content of the pituitary below that in normal rats [42].

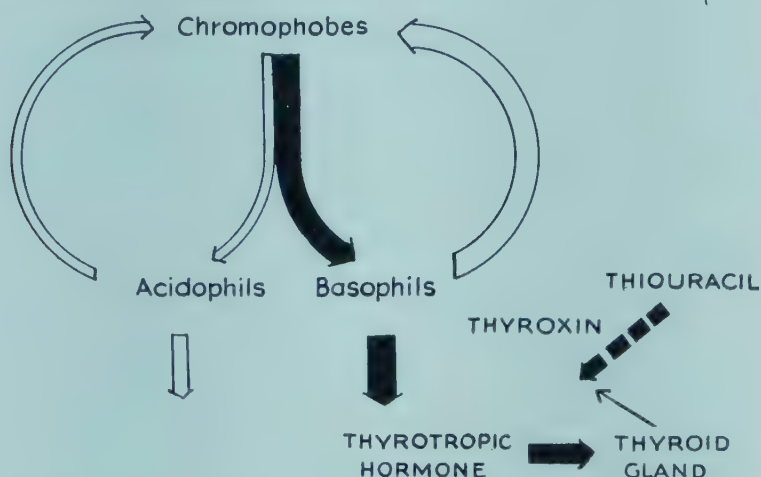


FIG. 15.—THE EFFECT OF THIOURACIL ON THE THYROID AND ANTERIOR PITUITARY GLAND

Williams and others [15], in some patients under thiouracil treatment, noted retention of sodium and chloride associated with hypernatraemia and hyperchloraemia, and a decrease in the carbon-dioxide combining power suggestive of increased adrenocortical activity. The decreased nitrogen, creatinine and creatine excretion is probably connected with hyperfunction of the androgenic element of the adrenals. No change, however, occurred in the 17-ketosteroid excretion and gluconeogenesis.

## Iodine

Iodine exerts a protective effect on the thyroid hormone and leads to considerable storage of thyroxine. It is only after the thyroid hormone stored in the body has been used up that thiouracil can exert its action; and for this reason patients who have been iodinated before thiouracil treatment maintain a raised metabolism for a longer time [16-19].

The rate at which metabolism falls to normal under thiouracil treatment depends upon the available stores of mobilizable thyroxine [17] the presence of which is responsible for the latent period after the institution of thiouracil treatment. In patients treated with

thiouracil the thyroid tissue shows a subnormal avidity for inorganic iodine, more than normal amounts of the administered iodine being excreted in the urine [16].

Iodine tends to produce involution of the thyroid gland and counteract the hyperplasia produced by thiouracil [20]. It is claimed that goitres of patients given both iodine and thiouracil become smaller, firmer and less vascular [17].

#### THERAPEUTIC EFFECTS

Relief of symptoms is usually obtained 2-4 weeks after the beginning of treatment [8, 11, 17-19, 24; 26]. A delayed response occurs in patients who have been treated with iodine before the institution of thiouracil treatment [18, 19]. Thyroid function is lowered to a degree which depends on the dosage of thiouracil.

It is yet too early to say whether thiouracil produces a permanent relief of symptoms, although preliminary reports are very promising. Eaton [22] reported seven cases which, after cessation of thiouracil treatment, remained well up to the date of publication, i.e. for from  $3\frac{1}{2}$  to 8 months. Astwood [28] reported sustained remission in cases in which thiouracil treatment was not discontinued until metabolic equilibrium had been maintained for 6-9 months. Himsworth's patients were off treatment up to 12 months without showing signs of recurrence [19]. Sustained remission after cessation of treatment is also reported by Watson [35] and others [43, 44]. On cessation of treatment sustained remission of the thyrotoxicosis was more apt to occur the longer the thiouracil had been given [44].

According to Himsworth and others [50] there is no appreciable difference in the condition of the patients treated with thiouracil and those subjected to subtotal thyroidectomy. The death rate due to subtotal thyroidectomy is 2.4 per cent [51] against 0.45 per cent with thiouracil treatment [52]. Thyroidectomy is advocated when tracheal obstruction is present, when a drug reaction occurs, and when the patient is unable or unwilling to attend regularly for an indefinite period [50].

#### In Pregnancy

Thiouracil had no untoward effects when given during pregnancy, except that in one of Eaton's patients who received the drug until confinement the child had an enlarged thyroid gland [22]. A pregnant woman treated with thiouracil, 200 mg. on alternate days, suddenly died of unknown cause. The thyroid of the foetus was enlarged and hyperplastic as compared with a presumably normal gland from a



premature infant and showed histological evidence of considerable functional activity. It resembled the gland of an adult receiving too much thiouracil [45]. That thiouracil passes the placental membrane has recently been demonstrated in the pregnant rat. Pregnant rats which had been given a diet containing 2-thio-4-*n*-propyluracil produced fetuses which, mixed in the food of normal rats, brought about hyperplasia of their thyroid glands.

### **Exophthalmos and Goitre**

Thiouracil does not, according to most workers, bring about improvement of exophthalmos and goitre [19, 21, 35]. Some workers [33, 37], however, report decrease of exophthalmos in a large proportion of cases.

### **Diabetes**

Hyperthyroidism is often accompanied by diabetes, and in such cases treatment with thiouracil results in increased glucose tolerance as ascertained by the insulin requirements [33, 37]. Apparently only those cases of diabetes benefit which are associated with secondary hyperthyroidism [47]. In rats the administration of thiouracil causes marked increase in liver glycogen, probably due to diminished glycogenolysis [48].

### **Auricular Fibrillation**

Auricular fibrillation, associated with thyrotoxicosis, usually disappears in most patients without other treatment than the administration of thiouracil [33, 37].

### **Angina Pectoris**

Treatment with thiouracil proved effective in 7 out of 10 patients with angina pectoris, 4 of whom became entirely symptom-free during treatment [27].

### **Congestive Heart Failure**

Cases of severe congestive heart failure showing no response to the usual treatment (bed-rest, digitalis and mercurial diuretics) were given 2-thiouracil 1 to 2 grams a day over long periods. Life may be prolonged both in the low-cardiac-output cases (valvular and hypertensive heart disease) and in the high-cardiac-output cases (heart failure with emphysema).

In the low-output group the arterio-venous oxygen difference may decrease as well as the resting oxygen consumption. The work of the

heart may be unchanged, but output is maintained at a lower venous pressure, and in relation to oxygen consumption cardiac output is relatively increased.

Cases of high-cardiac-output heart failure with emphysema may improve sufficiently to leave hospital in spite of permanent reduction of arterial oxygen saturation [49].

#### TOXIC REACTIONS

The following toxic reactions to thiouracil treatment are due partly to idiosyncrasy and partly to overdosage:

Agranulocytosis [19-23].

Thrombopenia [23].

Swelling of the submaxillary salivary glands [20, 21].

Jaundice: drug fever [21].

Oedema of the legs, urticaria, morbilliform rash, allergic arthritis, vomiting, fever, diarrhoea [20].

Agranulocytosis, the most dangerous complication, has mostly occurred as a result of overdosage. No untoward effects have been noted with small doses [22, 18], and toxic reactions usually regress with decrease in dosage [24].

A study of 1,091 patients treated with thiouracil, 0.5-0.8 gram daily for 3-7 weeks or until the B.M.R. was normal, following which the dose was reduced, revealed the following data:

1. Death attributable directly to the drug: 0.5 per cent.
2. Agranulocytosis: 1.8 per cent.
3. Death rate in agranulocytosis: 26 per cent.
4. Leucopenia without agranulocytic angina: 3 per cent.
5. Fever: 5 per cent.
6. Glandular enlargement: 5 per cent.
7. Miscellaneous reactions: 2 per cent.

This indicates that 1 in 10 patients in whom treatment was started had to have the treatment stopped through the appearance of a reaction which could not be handled safely without discontinuance of the drug. On the basis of the figures presented, approximately 2 per cent of the patients treated may be expected to develop agranulocytosis. The most dangerous period from the point of view of leukocyte depression is from 4 to 8 weeks after initiation of treatment [53].

Winkle and co-workers [54] present a survey of 5,745 cases treated with daily doses of 0.4 gram thiouracil initially and thereafter with 0.1

or 0.2 gram daily as a maintenance dose. According to their figures, approximately 13 per cent of all cases can be expected to show some adverse reaction to thiouracil therapy, and agranulocytosis occurs in about 2.5 per cent. No relationship to the dose of thiouracil was apparent.

The addition of 5 per cent solubilized liver to the diet almost completely prevented the development of neutropenia in the thiourea-fed rats. Waisman and Elvehjem suggested that the corrective factor in liver was folic acid, a view which has been confirmed by Daft and Sibrell's use of crystalline folic acid. The use of folic acid or liver is thus suggested for hyperthyroid patients treated with thiourea derivatives [25]. According to Williams and co-workers, however, the routine administration of special capsules of liver powder containing more folic acid than usual did not afford any definite protection against the development of toxic reactions [55]. Fishberg and Vorzimer [37] advocate the use of pyridoxine (vitamin B<sub>6</sub>) in prophylactic doses of 150 mg. daily by mouth; or 200 mg. intravenously in cases in which a severe drop in the leucocyte count has taken place. Better results are obtained with massive doses of penicillin (500,000 units per day) in the treatment of agranulocytosis [54, 56], and the use of streptomycin has been suggested in cases which do not respond to penicillin [57].

### Hyperplasia

Thiouracil often produces hyperplasia of the thyroid gland [7, 8, 12, 13, 16, 20, 21]. The degree of hyperplasia apparently depends on the dosage [58] of thiouracil, for it is this that determines whether the amount of thyroxin produced by the thyroid shall be sufficient to control the thyrotrophic function of the anterior pituitary lobe. To prevent thyroid hyperplasia thiouracil and iodine should be given in moderate doses [20, 17]. On the basis of experiments in animals [42] and humans [59, 60, 61] it is suggested that in woman the additional administration of oestrogens with thiouracil may prevent thyroid hyperplasia. No increase in the size of the gland due to thiouracil was noted by Fishberg and Vorzimer [37].

### Recommended Dosage

The usual dose of thiouracil is 0.5–0.6 gram daily for 2–3 weeks until the B.M.R. returns to normal, followed by a maintenance dose of 0.1–0.2 gram daily [17, 18, 19, 21, 22, 24, 26, 27, 29]. McGavack and others [24] found that 0.1 gram daily did not suffice in their cases to prevent recurrence of thyrotoxicosis.



In general, however, the lower dose of 0.1 gram daily is recommended for maintenance [19, 21, 27, 28]. Himsworth [19] advocates 0.6 gram daily until the B.M.R. falls to about + 10; and thereafter the dose is dropped to 0.1–0.2 gram daily for maintenance. McGavack and others [24] recommend the following treatment schedule:

B.M.R. from + 50: Thiouracil 0.8–1 gram daily (not longer than for 5–10 days).

B.M.R. between + 30 and + 50: Thiouracil 0.6 gram daily (for 2–3 weeks when the B.M.R. usually falls to normal).

B.M.R. less than + 30: Thiouracil 0.4 gram daily (for 2–3 weeks when the B.M.R. usually falls to normal).

Maintenance: Thiouracil 0.2 gram (or less) daily.

### **B.M.R.**

The rate at which metabolism falls to normal under thiouracil is governed chiefly by the available stores of mobilizable thyroxin, a delayed response occurring after pre-treatment with iodine [17]. When the B.M.R. has reached the normal level it falls no further, although the dose of thiouracil remains unchanged [19]. Increased dosage of thiouracil is valueless and even has positive disadvantages. It is not more effective than the smaller dosage, for the excess is apparently excreted in the urine [21, 27]; furthermore, it provokes increased production of the anterior pituitary thyrotrophic hormones and thus causes enlargement of the goitre; and it may produce one or more of the toxic reactions referred to on p. 226.

The dose of thiouracil should therefore be decreased without anticipating the appearance of a subnormal B.M.R. The dose should be progressively diminished until there are indications for adjustment.

### **Pre-operative Treatment**

Patients prepared for thyroidectomy by a course of thiouracil experience less severe thyrotoxic manifestations before, during and after the operation than do patients prepared with iodine, but their thyroid glands sometimes bleed more [20] and show moderate hypertrophy and marked hyperplasia [16]. Iodine causes involution of the thyroid gland, and the goitres of patients given both iodine and thiouracil become smaller, firmer and less vascular [17]. Iodine administered 3 weeks after treatment with thiouracil greatly decreases the technical difficulties of subtotal thyroidectomy.

Experience indicates that subtotal thyroidectomy can be done with greatly reduced risk if preoperative treatment with thiouracil is carried

ut. Hyperthyroid reactions during anaesthesia and after operation can be eliminated completely. The death rate is 0.5 per cent [62] against 2.4 per cent for subtotal thyroidectomies not preceded by thiouracil treatment.

#### METHYL THIOURACIL

Recent investigations have revealed the effects of methyl thiouracil in thyrotoxicosis. In one series of 16 cases [30] the dose administered was 0.2 gram, stepping up 0.2 gram every day or every other day, to an effective daily limit of 0.8 gram. The maintenance dose was 0.05–0.2 gram. All cases responded satisfactorily, but minor toxic symptoms were observed, most of the patients who were not given liver showing a depression of the leucocyte count. All the patients had an eosinophilia, with counts ranging from less than 50 to 200 or more per cubic mm. Half an ounce of proteolysed liver [Evans] by mouth daily did not prevent the eosinophilia.

Recent clinical data indicate that methyl thiouracil has superseded thiouracil in the treatment of thyrotoxicosis [63]. Its advantages over thiouracil are a reduction in the lag period, a reduction in the chances of producing thyroid hyperplasia [64] or decreased liability to produce toxic effects [65], and a smaller maintenance dose (0.1 to 0.025 gram) [66].

#### TETRAMETHYL THIOUREA [31]

The recommended dosage for tetramethyl thiourea is 0.2 gram at 4-hourly intervals until the basal metabolic rate becomes normal, and then a maintenance dose of 0.2 or 0.1 gram daily. Patients so treated were found to have a higher blood content of the drug than those treated with thiouracil in a like manner. Granulopenia was not observed in any of the patients.

#### DIETHYL THIOUREA

Diethyl thiourea was used in the treatment of 4 thyrotoxic patients. The results were unsatisfactory and the daily dose of 0.6 gram proved highly toxic.

#### PARAXANTHINE [32]

It has been suggested that paraxanthine may antagonize the effects of preformed thyroxine [38], unlike thiouracil which acts by inhibiting its production. But in all studies, experimental and clinical, paraxanthine has proved ineffective.

## AMINOTHIAZOL

The effective dose is about 0.4 gram in cachets or tablets each containing 0.01 gram of aminothiazol. Treatment is started with 0.6 or even 0.8 gram, after improvement has occurred, continued with a maintenance dose of 0.3 or 0.2 gram or even less. This dosage is divided into 3-6 portions. Satisfactory and often striking results are reported. Subjectively the effect occurs very quickly, usually within 3 to 7 days, the patient feeling relief from most of his functional disturbances. On the whole the drug is well tolerated. Complications which occurred in 11.6 per cent of cases, consisted of drug fever, erythema, urticaria, digestive intolerance, lumbar pain and oliguria. No blood changes have been observed [67].

## THIOBARBITONE

Thiobarbitone (5,5-diethyl-2-thiobarbituric acid) differs from barbitone only to the extent that one oxygen atom is replaced by an atom of sulphur. It is only moderately active as a sedative or hypnotic [68].

Thiobarbitone is more active than thiouracil in inhibiting thyroid function in man. Given at 8- or 12-hour intervals the minimal initial dosage of thiouracil which will control the majority if not all cases of hyperthyroidism has proved to be 0.4 gram daily, though 0.2 gram has been sufficient in some cases.

Astwood [69] reports that 0.2 gram of thiobarbitone has not failed to be effective and 0.1 gram was adequate in all instances. Thus it would seem that thiobarbitone is a little more than twice as effective as thiouracil. The average maintenance dose of thiouracil is 0.2 gram daily in 2 doses at 12-hour intervals, while the maintenance dose of thiobarbitone averages 0.1 gram daily or somewhat less. The data also show that a single oral dose of thiobarbitone has a more prolonged action than a single dose of thiouracil. It is now well recognized that the effectiveness of thiouracil is greatly diminished if the doses are spaced at 24-hour intervals instead of at 8 or 12 hours [70]. Thiobarbitone is more toxic than thiouracil when the minimal effective dose is exceeded.

## PROPYLTHIOURACIL

Propylthiouracil is undergoing clinical investigation for the treatment of thyrotoxicosis. In man it is about 5 times as active as thiouracil [71] and 4 times as potent as methyl thiouracil [72]. The response to propylthiouracil is not more rapid than that to methyl thiouracil [72]. The use of this derivative is less attended by untoward side effects



than thiouracil [73]; and it is less liable to cause agranulocytosis, which in thiouracil-treated cases has caused 30 deaths [74]. Its greater safety [71-8] makes it thus superior to any other antithyroid drug. The recommended initial dosage is 150 mg. every 8 hours for severe cases and 50 mg. twice daily for milder cases [75] until all manifestations of the disease have been controlled. Twenty-five to seventy-five mg. daily are advocated as maintenance doses [71-3].

### YOHIMBINE

More than 25 years ago, an aphrodisiac action was ascribed to yohimbine, an alkaloid of the formula  $C_{22}H_{30}N_2O_4$  derived from the bark of the tree *Pansinystalia yohimba* of the Cameroons and French Congo. Its action has been related to the production of pelvic and genital hyperaemia. Recently, however, evidence has been supplied by Fugo and Gross [1] that the aphrodisiac action of yohimbine is due to its pituitary-stimulating action resulting in the release of luteinizing hormone.

Fugo and Gross treated adult female rats for 40-101 days with daily doses of from 1 to 4 mg. of yohimbine hydrochloride. In the animals which received the lower dose of 1-2 mg. oestrus was prolonged from 3 to 9 days; those which received the higher dose of 3-4 mg. developed a condition resembling pseudopregnancy.

Similar treatment of adult castrated rats did not produce vaginal cornification, which suggested that the oestrus produced in the intact rat was not due to hyperaemia; and the failure of yohimbine to produce oestrus in the hypophysectomized rat seemed to prove that its gonadotrophic action was mediated by the pituitary.

The authors further implanted immature ovaries devoid of corpora lutea into adult castrated male rats and found that normal corpora lutea appeared when the animals were treated with yohimbine hydrochloride. Immature female rats did not respond to yohimbine hydrochloride 1 mg. daily, probably because at that age the pituitary was not ready to respond to stimulation.

On the basis of the experimental evidence the authors concluded that yohimbine hydrochloride stimulates the release of excessive amounts of anterior pituitary hormones, especially of the luteinizing fraction.

The clinical dosage recommended is 100 mg. daily [2].

In addition to its anterior pituitary-lobe stimulating effect Fugo [3] found that yohimbine hydrochloride, when administered to normal dogs, produces an anti-diuresis which presumably results

from the release of an anti-diuretic principle by the posterior lobe. When the posterior lobes were extirpated or the dogs were completely hypophysectomized, administration of yohimbine failed to relieve polyuria. On the contrary, in animals with diabetes insipidus, the volume of urine increased, suggesting that some diuretic principle had been released from the anterior lobe. This view gains support from the observation that no significant change in the volume of urine occurred in completely hypophysectomized dogs.

Results which apparently conflict with the view that yohimbine hydrochloride acts by stimulating the pituitary have been recorded by Sulman and Black [4]. In a very detailed study on 120 immature female and male rats, 35 adult female rats and 20 adult spayed female mice, subcutaneous injections of yohimbine hydrochloride in a dosage of 0.3–5 mg. per day failed to show a pituitary-stimulating effect. Sulman and Black's results, however, were obtained by a different methodology and approach from those of Fugo and Gross and do not conclusively prove that yohimbine hydrochloride is devoid of pituitary-stimulating action. The problem obviously calls for further investigation.

## BIBLIOGRAPHY

### THIOURACIL

1. MACKENZIE, G. B. and C. J., and MCCOLLINS, E. V. *Science*, **94**, 518, 1941.
2. KENNEDY, T. H. *Nature*, **150**, 233, 1942.
3. MACKENZIE, C. J. and J. B. *Endocrinol.*, **38**, 185, 1943.
4. ASTWOOD, E. B., SULLIVAN, J., BISSELL, A., and TYSLOWITZ, R. *Endocrinol.*, **32**, 210, 1943.
5. ASTWOOD, E. B. *J.A.M.A.*, **122**, 78, 1943.
6. WILLIAMS, R. H., and BISSELL, G. V. *New England J. Med.*, **229**, 97, 1943.
7. HIMSWORTH, H. P. *Lancet*, **245**, 465, 1943.
8. GARDINER-HILL, H. *Practitioner*, **152**, 94, 1944.
9. FRANKLIN, A. L., LERNER, S. R., and CHAIKOFF, I. L. *Endocrinol.*, **34**, 265, 1944.
10. ASTWOOD, E. B., and BISSELL, A. *Endocrinol.*, **34**, 282, 1944.
11. KESTON, A. S., GOLDSMITH, E. D., GORDON, A. S., and CHARIPPER, H. A. *J. Biol. Chem.*, **152**, 241, 1944.
12. PEACOCK, W. *Endocrinol.*, **34**, 245, 1944.
13. RAWSON, R. W., and PASCHKIS, K. E. *J.A.M.A.*, **128**, 65, 1945.
14. WILLIAMS, R. H., WEINGLASS, A. R., BISSELL, G. W., and PETERS, J. B. *Endocrinol.*, **35**, 317, 1944.
15. WILLIAMS, R. H., BISSELL, G. W., JANDORF, B. J., and PETERS, J. B. *J. Clin. Endocrinol.*, **4**, 58, 1944.

16. ROULON, W., RAWSON, R. D., EVANS, J. H., MEANS, W. C., PEACOCK, J., LERMAN, J., and CORTELL, R. E. *J. Clin. Endocrinol.*, **4**, 1, 1944.
17. ASTWOOD, E. C. *J. Clin. Endocrinol.*, **4**, 229, 1944.
18. BARTELS, E. C. *J.A.M.A.*, **125**, 24, 1945.
19. HIMSWORTH, H. P. *The Clin. J.*, **74**, 97, 1945.
20. WILLIAMS, R. H., and CLUTE, H. M. *J.A.M.A.*, **128**, 65, 1945.
21. DONALD, J. B., and DUNLOP, D. M. *Brit. Med. J.*, 4386, 117, 1945.
22. EATON, J. C. *Lancet*, **148**, 171, 1945.
23. NEWCOMBE, P. B., and DEANE, E. W. *Lancet*, **236**, 179, 1944.
24. MCGAVACK, T. H., GERL, A. J., VOGEN, M., and SCHWIMMER, D. *J. Clin. Endocrinol.*, **4**, 249, 1944.
25. GOLDSMITH, E. D., GORDON, A. S., FINKELSTEIN, G., and CHARIPPER, H. A. *J.A.M.A.*, **125**, 847, 1944.
26. RAAB, W. *J.A.M.A.*, **128**, 249, 1945.
27. HIMSWORTH, H. P., and JOLL, C. A. *Proc. Roy. Soc. Med. London*, **37**, 693, 1944.
28. ASTWOOD, E. B. Ann. Meet. Assn. for the Study of Internal Secretions, Chicago, 1944.
29. NUSSEY, A. M. *Brit. Med. J.*, 4379, 745, 1944.
30. LEYS, DUNCAN. *Lancet*, **248**, 461, 1945.
31. WILLIAMS, R. H. *J. Clin. Endocrinol.*, **5**, 210, 1945.
32. *Ibid.*, **5**, 217, 1945.
33. MCGAVACK, T. H., GERL, A. J., MORTON, J. H., VOGEL, M., and SCHWIMMER, D. *J. Clin. Endocrinol.*, **5**, 259, 1945.
34. BAUMAN, E. J., and MARINE, D. *Endocrinol.*, **36**, 400, 1945.
35. WATSON, E. M. *J. Clin. Endocrinol.*, **5**, 273, 1945.
36. KING, B. T., and ROSELLINI, L. J. *J.A.M.A.*, **129**, 267, 1945.
37. FISHBERG, E. H., and VORZIMER, J. *J.A.M.A.*, **128**, 915, 1945.
38. CARTER, G. S., MANN, F. G., HARLEY-MASON, J., and JENKINS, G. N. *Nature*, **151**, 728, 1943.
39. REVENO, W. S. *J. Clin. Endocrinol.*, **5**, 403, 1945.
40. AZIMOV, G. I., and ALTMAN, A. D. *Comptes Rend. de l'Acad. des Sc. de l'U.S.S.R.*, **20**, 621, 1938.
41. SMELSER, G. K. *Endocrinol.*, **34**, 39, 1944.
42. MEITES, J., and TURNER, C. W. *Proc. Soc. Exper. Biol. & Med.*, **64**, 488, 1947.
43. BARR, D. P., and SHORR, E. *Ann. Int. Med.*, **23**, 754, 1945.
44. WILLIAMS, R. H. *J. Clin. Endocrinol.*, **6**, 1, 1946.
45. DAVIS, L. J., and FORBES, W. *Lancet*, **2**, 740, 1945.
46. FREIESLEBEN, E., and KJERULF-JENSEN, K. *J. Clin. Endocrinol.*, **7**, 47, 1947.
47. REVENO, W. S. *Am. J. Med. Sci.*, **211**, 174, 1946.
48. MAY, L. G., MOSELY, R. W., and FORBES, J. C. *Endocrinol.*, **38**, 147, 1946.
49. SHARPEY-SHAFER, E. P. *Brit. Med. J.*, 4484, 888, 1946.
50. HIMSWORTH, H. P., MORGANS, M. E., and TROTTER, W. R. *Lancet*, **1**, 241, 1947.



51. MORLEY, J. *Lancet*, **2**, 690, 1944.
52. FOWLER, E. F., and COLE, W. H. *Surg. Gynec. & Obst.*, **84**, 350, 1947.
53. MOORE, D. *J.A.M.A.*, **130**, 315, 1946.
54. WINKLE, VAN W., JR., HARDY, S. M., HAZEL, G. R., HINES, D. C., NEWCOMER, H. S., SHARP, E. A., SISK, W. N. *J.A.M.A.*, **130**, 343, 1946.
55. WILLIAMS, R. H., CLUTE, H. M., ANGLE, T. I., and KENNEY, F. R. *J. Clin. Endocrinol.*, **6**, 23, 1946.
56. BEIERWALTER, W. H., and STURGIS, C. C. *Am. J. Med. Sc.*, **212**, 513, 1946.
57. SELIGMAN, B., and WEINTROB, M. *J. Clin. Endocrinol.*, **7**, 219, 1947.
58. ENGLE, E. R., and ARANOW, H., JR. *Endocrinol.*, **38**, 325, 1946.
59. GESSLER, C. *Arch. Internat. de Pharmacodyn. et de Therap.*, **54**, 263, 1936.
60. SHERWOOD, T. C., SAVAGE, M., and HALL, J. F. *Am. J. Physiol.*, **105**, 241, 1933.
61. FAEBMAN, A. A. *J. Clin. Endocrinol.*, **4**, 17, 1944.
62. BARTELS, E. C., and BELL, G. O. *West. J. Surg., Obst. & Gynec.*, **55**, 39, 1947.
63. POATE, H. R. G. *Med. J. of Australia*, **2**, 789, 1946.
64. GLOCK, G. E. *Brit. J. Pharmacol. & Chemother.*, **1**, 127, 1946.
65. FRISK, A. R. *Hygiea, Nordisk Medicin. Gothenburg*, **31**, 1575, 1946.
66. WILSON, A. *Lancet*, **1**, 640, 1946.
67. PERRAULT, M., and BOVET, D. *Lancet*, **250**, 731, 1946.
68. GRUHZIT, O. M., DOX, A. W., ROWE, L. W., and DODD, M. D. *J. Pharmacol. & Exper. Therapy*, **60**, 125, 1937.
69. ASTWOOD, E. B. *J. Clin. Endocrinol.*, **5**, 345, 1945.
70. *Ibid.*, **4**, 229, 1944.
71. ASTWOOD, E. B., and VANDER LAAN, W. P. *J. Clin. Endocrinol.*, **5**, 424, 1945.
72. WILSON, A., and GOODWIN, J. *Lancet*, **252**, 669, 1947.
73. ASTWOOD, E. B., and VANDER LAAN, WILDER P. *New Eng. Med. Center*, **8**, 143, 1946.
74. ASTWOOD, E. B. *Jackson Clin. Bull.*, **8**, 148, 1946.
75. ASTWOOD, E. B., and VANDER LAAN, W. P. *Ann. Int. Med.*, **25**, 813, 1946.
76. KJERULF-JENSEN, K., and MEULENGRACHT, E. *Nordisk Medicin*, **32**, 2809, 1946.
77. REVENO, W. S. *J.A.M.A.*, **133**, 1190, 1947.
78. MCCULLAGH, E., PERRY, R., EDWARD, J., and SCHNEIDER, R. *Cleveland Clin. Quart.*, **13**, 232, 1946.

#### YOHIMBINE

1. FUGO, N. W., and GROSS, E. G. *Endocrinol.*, **31**, 529, 1942.
2. HAMBLIN, E. C. *J. Clin. Endocrinol.*, **3**, 52, 1943.
3. FUGO, N. W. *Endocrinol.*, **34**, 143, 1944.
4. SULMAN, F., and BLACK, R. *Endocrinol.*, **31**, 70, 1945.

## VITAMIN-ENDOCRINE RELATIONSHIPS

## SIMILARITY

THE vitamins and hormones have many physiological and pharmacological properties in common. The vitamins, like the hormones, are active in minute doses and act upon the body like catalysts. For example, nicotinic acid, thiamine and riboflavin are constituents of several enzyme systems, and ascorbic acid is invaluable in oxidation-reduction processes.

## DIFFERENCES

One of the more striking differences between vitamins and hormones lies in the fact that the former, unlike the latter, are of exogenous origin, or are produced in the body by extraneous physical influences, as, for example, vitamin D by ultra-violet radiation. There are, however, some exceptions to this rule. For example, dogs and rats do not depend upon dietetic supplies of vitamin C, but form it themselves.

Another outstanding difference is that vitamins are, in general, chemically more stable and simpler compounds than hormones. Unlike the hormones, they are not destroyed in the gastro-intestinal tract.

In their biological action, some vitamins enhance certain glandular functions, whereas others are antagonistic. Thus, vitamin D resembles thyroxin in its influence on phosphorus metabolism (increase of inorganic phosphorus in the blood), and the parathyroid hormone in its influence on calcium metabolism.

Although both vitamin D and the parathyroid hormones raise blood calcium, they differ in their action, for the parathyroid hormone acts upon the bone while vitamin D influences the permeability of the intestinal epithelium and thus probably leads to better absorption of calcium and phosphorus [1].

## ANTAGONISTIC RELATIONSHIP

The question of vitamin and hormone relationships has recently been reviewed by Korenchevsky, Hall and Klapham [2]. They maintain that there is no true antagonism between vitamins and the thyroid hormone, but that when metabolism is increased, as by excess of thyroid hormone, the tissues develop an increased need for vitamins, particularly A, B, C and D. This may be due to increased oxidation, or to increased destruction or excretion of vitamins. In short, the thyroid gland, in raising the total energy output, at the same time increases the vitamin requirements of the body. They demonstrated,

in experiments on rats, that small doses of thyroid or thyroxin, given with a diet containing adequate doses of vitamins, exert anabolic as well as catabolic effects. Although the weight of the muscles was much reduced, the total body weight was kept up as a result of hypertrophy of many of the internal organs. Histologically, there was evidence of depressed thyroid function, and hypertrophy of the heart, liver and kidneys. The effects resembled those obtained by androgen administration. On the basis of this work the authors suggest cautious trials of thyroid hormone, together with vitamins or sex hormones, in diseases of the liver, kidney or heart, or in conditions calling for stimulation of the ovaries, adrenals or spleen.

When the animals were given large toxic doses of either thyroid or thyroxin plus their usual diet, predominant catabolic rather than anabolic effects were reflected in loss of weight in most of the organs studied. Depression of thyroid function was apparent, and there was hypertrophy of the heart and adrenals. Extra vitamin B supplies, even when associated with toxic doses of thyroid, produced gain of weight; and some catabolic effects on specific organs, characteristic of thyrotoxicosis, were reversed and became anabolic instead.

Halibut- and cod-liver oils had a slightly depressing effect on the thyroid function, which may have been due to the excessive iodine content of the oil. Vitamins A and D did not appear to effect the reaction of the body to large doses of thyroid; vitamins B and C, however, appeared slightly to decrease the effect of the hormone as a stimulus of hypertrophy in the liver and kidneys.

The authors concluded that the reversal of some, though not all, of the usual effects of thyrotoxicosis by the administration of extra vitamins does not prove that the vitamins and hormones are mutually antagonistic, but rather that the extra vitamins made good a relative deficiency produced by the thyroid and complicating its effects.

## VITAMIN A

### Thyroid

The conversion of carotene to vitamin A is impaired with thyroid deficiency. Supplements of vitamin A administered to rats receiving a diet devoid of vitamin A prevented the appearance of xerophthalmia in both control and thyroidectomized animals. However, supplements of carotene prevented the ocular changes only in the control rats and not in thyroidectomized rats. The mortality and changes in weight of the thyroidectomized animals receiving carotene also indicated a decreased utilization of carotene [3].



Overaction of the thyroid increases the conversion of carotene above the normal. Moore [6] found larger stores of vitamin A in the livers of patients who had died from thyrotoxicosis than were present in other human livers. Fasold and Heidemann [7] found that thyroidectomized goats secrete yellow milk containing much carotene, whereas normal goats secrete colourless milk containing much vitamin A. Destruction of vitamin A in the body is not increased by thyroxin.

Evidence of the damping effect of vitamin A on thyroid activity was given by McCarrison [8], who found that cod-liver oil delayed the metamorphosis of tadpoles. This has since been confirmed by the use of a purer vitamin A preparation.

Wegelin [52] found that administration of vitamin A checked the loss of liver glycogen caused by thyroxin.

Many workers have shown by animal experiment that the administration of vitamin A reduces the increased metabolism caused by thyroxin; but the fact that this action is even more marked when thyroxin is given as well suggests that vitamin A and thyroxin, far from being antagonistic, actually reinforce each other in their action on the thyroid [9-12].

According to some workers changes in the endocrine glands due to lack of vitamin A are secondary to a primary change in the pituitary. The amount of thyrotrophic hormone in the anterior pituitary appears to be low in rats on a high vitamin A diet, and high in rats on a deficient diet [13] (see p. 50).

### Other Glands

The adrenals sometimes store large amounts of vitamin A [53, 54].

Larger amounts of vitamin A are required during puberty than in periods of normal growth. Deficiency of the vitamin causes sterility in female rats [55]. Excessive amounts of carotene are reported to stop oestrus and a desire to mate [56].

The pancreas and thymus react to vitamin A deficiency by a decrease in size.

## VITAMIN B<sub>1</sub> (ANEURIN, THIAMINE)

### Thyroid

There is a striking resemblance between experimental B<sub>1</sub>-avitaminosis and myxoedema. Both cause cardiac changes and lowering of the temperature and basal metabolism; in extreme vitamin B<sub>1</sub> deficiency, the thyroid gland atrophies [57]. Williams and Kendall [15] found that

thyroxin is less effective in promoting metabolic activity in vitamin B<sub>1</sub> deficient than in normal animals.

Drill [42] examined the livers and kidneys of rats receiving 100 mg. of desiccated thyroid gland and found that they contained less vitamin B than those of normal controls, even when the hyperthyroid animals were given 500  $\mu$ g. of vitamin B in addition to the basic diet.

Using synthetic vitamin B, Drill and Hayes [47] showed that this prevents the loss of liver glycogen produced in rats and dogs by administration of thyroid or thyroxin. A high vitamin B diet in thyroxin-treated dogs delays the advance of liver damage as determined by the bromosulphaleine test. Furthermore, the pulse rate and temperature in experimental thyrotoxicosis are influenced by the amount of thiamine in the diet (see p. 50).

### **Adrenal Cortex**

It seems that the adrenal cortex plays a part in avitaminosis-B<sub>1</sub> for it hypertrophies in beri-beri; and adrenalectomized animals tolerate vitamin B<sub>1</sub>-free diets less well than the controls.

### **Vitamin B and Carbohydrate Metabolism**

The rôle of the vitamin B complex in carbohydrate metabolism is illustrated by recent studies of Gaebler and Mathies [4]. Losses of weight and nitrogen, and marked glycosuria, occurred in some depancreatized dogs during deficiency of water-soluble vitamins, even though the food intake and the insulin dose remained constant, and choline or other lipotropic factors were supplied. Control of diabetes was re-established when thiamine, riboflavin, nicotinic acid, pyridoxin and pantothenic acid were added to the diet. Pyridoxin and pantothenic acid, separately and together, diminished glycosuria and improved the nitrogen balance in experiments in which they were the only variables.

Pantothenic acid deficiency in dogs greatly diminishes the rates of absorption of carbohydrate and protein, without notably altering total digestion and absorption [5]. Thiamine and pyridoxin favourably influence absorption in rats [14] and the suggestion has been made that pyridoxin is involved in conversion of protein to carbohydrate [21]. These effects would tend to increase glycosuria. But if the insulin supply is such that glucose absorbed or formed at normal rates is stored as glycogen until needed, then thiamine, riboflavin, pyridoxin, and pantothenate might all diminish glycosuria, for, apart from any



effects upon oxidation, these factors promote conversion of carbohydrate to fat [22] (see p. 50).

### Oestrogen

Oestrogens are inactivated by the liver. Partial hepatectomy in the spayed female rat reduces the amount of  $\alpha$ -oestradiol required to produce vaginal oestrus in 50 per cent of the animals when the oestrogen is injected either subcutaneously or intrasplenically. The reduction is greater when the oestrogen is administered by the intrasplenic route [37].

Zondek and co-workers [44] demonstrated that stilboestrol is less rapidly inactivated than oestrone by liver pulp *in vitro*. Within a given time it takes twice as much liver to inactivate stilboestrol as oestrone. This finding may explain the greater oral efficiency and the toxic side-effects of stilboestrol. In rats treated with large amounts of stilboestrol, the capacity of the liver to inactivate stilboestrol is increased.

The oestrogen-inactivating function of the liver is considerably impaired by vitamin B deficiency. In the rat, such deficiency decreases the ability of the liver to inactivate oestrone and alpha oestradiol [43]. In rats fed on a B-deficient diet, the vaginal response to oestrogen injected intrasplenically has been found to be greater than that of normally fed controls. When, however, the oestrogen is injected subcutaneously, the vaginal response to oestrogen is decreased. This latter finding is interpreted as due to a reduction in the end-organ response to oestrogenic hormone.

Deficiency of vitamin B complex produces impairment of liver function only in respect of oestrogens; the liver continues to inactivate androgens, thus leading to a serious alteration of the oestrogen-androgen equilibrium [45]. One possible consequence of such an alteration is indicated by Lipschutz and his collaborators, who have shown that subserous fibroids can be produced by oestrogen, not only in the uterus, but also in other abdominal organs and in the abdominal wall. Fibroids thus produced can be prevented by the simultaneous administration of testosterone (or of other steroids having the androstene nucleus). An absolute rise in body oestrogen, not accompanied by a physiologically comparable rise in androgen, would favour the production of such tumours. Impaired oestrogen inactivation and vitamin B deficiency form part of a vicious circle, since increased amounts of oestrogen itself may cause vitamin B deficiency [49]. Signs of spontaneous exacerbations during the menstrual cycle were observed in B-deficient patients, and such



exacerbations could be produced by the administration of oestrogen [49].

Recent investigations [38, 46] shed some doubt on the rôle of the vitamin B complex in the inactivation of oestrogens (see p. 139).

### Male Sterility

The rôle of the vitamin B complex in male sterility has been shown by Biskind and others [16, 17]. Their observations indicate that deficient spermatogenesis may be related to faulty nutrition, perhaps even far more than hitherto suspected. Nutritional therapy, especially with vitamin B complex, either alone or in combination with vitamin E, was followed by improvement in the number, motility and morphology of sperms in previously infertile men. In other cases of sterility, in which initial specimens of spermatic fluid were apparently normal, treatment with vitamin B complex alone apparently restored fertility without producing significant detectable changes in the sperm.

Among 12 cases so treated, excluding 1 case of virtual azoospermia, there occurred 8 impregnations. The wife of one patient had a miscarriage at about the fourth month, but at the time of the publication [17] the remainder were approaching term or had been delivered of normal babies.

### VITAMIN B<sub>2</sub> (RIBOFLAVIN)

Chemically vitamin B<sub>2</sub> corresponds to flavin-phosphoric acid, and is a component of the so-called yellow respirator ferment [48]. It should be regarded as the co-ferment of the iron-free oxidation ferment. Its provitamin, flavin, is a yellow pigment found in whey, from which it is obtained.

Riboflavin must be phosphorylated before it can possess vitamin activity. The phosphorylation may occur as soon as it is absorbed from the intestinal wall, since preparations of intestinal mucosa can bring about the phosphorylation of riboflavin in the presence of phosphates. It was originally thought [58] that the adrenal cortex hormone was essential for phosphorylation, but later workers have shown that this view is incorrect, since phosphorylation is essentially normal after adrenalectomy [39, 40]. Riboflavin deficiency causes disturbances in certain aspects of rat's metabolism which resemble those seen in adrenal insufficiency. Administration of adrenal cortical hormone corrects these disturbances [59].

Riboflavin promotes the oxidation of carbohydrates, and also of amino acids, lactic acids and aldehydes.

## PANTOTHENIC ACID

Pantothenic acid is believed to be universally present in living tissue and is interrelated with the functions of plant respiration. It is a powerful stimulant to growth.

Rats on a diet deficient in pantothenic acid show a lethargic diuresis when water is given by stomach tube, and a decreased resistance to water intoxication. These defects are remedied by the administration of either calcium pantothenate or adrenal cortical hormones. In view of the reported adrenal pathology in pantothenic acid deficiency, the results are consistent with but do not prove the idea that adrenal hypofunction exists in this vitamin deficiency [59].

The effect of pantothenic acid deficiency has recently been further investigated by Deane and McKibbin [61].

Weanling male rats placed on a purified diet deficient in pantothenic acid grew poorly in comparison to their controls and presented other symptoms commonly ascribed to this deficiency. Moreover, their adrenal cortices became relatively large and the zonae reticularis and fasciculata were progressively drained of ketosteroids and gave cytological evidence of exhaustion. By the end of six weeks the zona fasciculata was entirely depleted of its hormone. While there were no apparent significant alterations in blood glucose and non-protein nitrogen, the severely deficient animals exhibited a decrease in liver glycogen as well as shrunken and often dying liver cells. After the fourth week of the deficiency the kidney tubule cells near the medulla likewise showed signs of degeneration. The thymuses of the deficient rats were atrophied in comparison with those of the controls. All of these symptoms are interpreted as indicating that a pantothenic acid deficiency acts as an "alarming agent" for the rat. Such agents cause the release of pituitary adrenotrophin which stimulates the adrenal to enlarge and increase its secretion of corticosterone. A deficiency of pantothenic acid is so severe an "alarming agent" that the adrenal becomes exhausted of corticosterone.

## FOLIC ACID

Folic acid is one of the latest factors of the B complex to be isolated and little information is yet available about it. It has been shown to increase the growth rate in rats [62] and to influence the colour of hair in these animals [63]. Since folic acid is to be found in the liver which inactivates oestrogens the influence of folic acid on oestrogens was investigated. Briefly the results suggested that folic acid inactivates oestrogens only to a moderate degree [64] and augments the oestrogenic effect in folic-acid-deficient chicks [50] (see p. 140).

## VITAMIN C (ASCORBIC ACID)

Vitamin C is present in high concentration in the adrenal cortex, adrenal medulla, corpus luteum and anterior lobe and pars intermedia of the pituitary [18], and it appears that special relations exist between vitamin C and these glands.

### Suprarenal Gland

The adrenal cortex and medulla are the main storage depots for vitamin C. The supposed association with oxidation-reduction processes in tissues suggests that it plays an important part in the metabolism of the adrenals. It is located in the region of the golgi apparatus of these cells of the adrenal cortex in which the biologically active 17-ketosteroids are present [19]. The injection of ACTH in rats and guinea pigs is followed by a prompt fall in the adrenal ascorbic acid [20]. The pigmentation of Addison's disease may be strikingly diminished by the oral or parenteral administration of vitamin C [23].

### Thyroid

The relationship between vitamin C and the thyroid is described on p. 51.

### Ovary

Biskind and Glick [60] found a high concentration of vitamin C in the corpus luteum, with a fall in the vitamin C content as the organ atrophies. They suggest a possible connection between vitamin C and progestin, for the vitamin C content of the corpus luteum appears to run parallel with the progestin content. Pillay [24] examined the urinary excretion of vitamin C of 11 women through 24 menstrual cycles and obtained some evidence of decreased excretion at the time of ovulation. Michaelson and others [25] examined the fasting vitamin C levels in the blood of 8 nurses during their menstrual cycle and found, in some cases, a sharp increase of plasma vitamin C during the middle of the cycle. In 4 of the 8 women studied, there was evidence of slight haemolysis in blood specimens taken during the peak of the plasma vitamin C.

Phillips [26, 27] demonstrated a marked increase in the plasma vitamin C level of cows the day they came into heat. Wisconsin workers found a close correlation between these peaks of plasma vitamin C and fertility. In some cases sterile cows, which showed no structural abnormalities of the reproductive tract, were made fertile by pre-coital intravenous injections of large amounts of vitamin C.



## VITAMIN D

Vitamin D insufficiency causes failure of calcium absorption from the gastro-intestinal tract, thus leading to a low serum-calcium level. This is compensated for by increased parathyroid function, which in turn restores the normal calcium level and lowers the serum-phosphorus level by increasing the urinary excretion of phosphate. The result is the usual finding of a normal calcium and low serum-phosphorus level [36]. On the other hand renal insufficiency associated with retention of serum phosphorus produces a negative calcium balance, which in turn induces increased parathyroid function with consequent decalcification of bone and a high serum-calcium level.

The parathyroid lowers the renal threshold for phosphates, and causes a fall in serum phosphate. The serum, being less saturated with calcium phosphate, develops an increased requirement for this compound. As a result, bone mineral is mobilized, unless as much calcium and phosphorus is ingested as is lost in the urine. Calcium is less rapidly excreted in the urine than phosphate, and its retention thus produces a higher blood-calcium level, with a tendency to deposit calcium in the soft tissues [51, 35] (see p. 60).

## VITAMIN E

The term vitamin E is applied to a group of naturally-occurring substances which possess the properties attributed to this vitamin. The biologically most active of these is alpha-tocopherol, and the less active are beta- and gamma-tocopherol.

The relationship of vitamin E to the endocrine glands has been extensively investigated with results which, on the whole, do not confirm the suggestion that the endocrine system is primarily responsible for the changes observed in vitamin E-deficient animals [28].

A diet devoid of vitamin E causes testicular degeneration in rats, but the progress is not arrested by vitamin E concentrates, either alone or in combination with extracts from human pregnancy urine or pregnant mare serum. Female rats fed on a vitamin E-deficient diet are able to conceive, but the foetus soon dies and is absorbed, as ascertained by observations on the weight curve of the pregnant female.

Vitamin E taken in the diet or in special preparations is deposited mainly in the organs concerned in procreation, the anterior pituitary lobe and the placenta (Zondek). Reports on the beneficial effect of vitamin E in sterility, habitual abortion, premature labour, muscular dystrophy and nervous disturbances are conflicting.

### Threatened Abortion

Currie [41] reported beneficial results in 90 per cent of cases of threatened abortion, and in 76 per cent of cases of habitual abortion treated with vitamin E. Shute obtained successful results in 31 of 46 cases of threatened abortion, but found no evidence that vitamin E, even in large doses, was of any value in the treatment of habitual abortion [29-31].

Premature detachment of the placenta can be foreseen by means of a blood-oestrogen assay. The signs disappear promptly if a potent preparation of vitamin E is administered at once. The vitamin must usually be given in increasing dosage as pregnancy proceeds, and its administration must continue up to the time of delivery. The investigation of women with threatening abortion revealed increasing response of their blood serum to proteolysis; but with vitamin E therapy the blood serum returned to normal. Shute concluded [30] that vitamin E and oestrogen exist in a sort of equilibrium during pregnancy. If there is too much oestrogen, the pregnancy is interrupted, though an excess of vitamin E does not appear to affect the pregnancy. In cases of threatened abortion, with premature partial separation of the placenta and a high anti-proteolytic factor (possibly oestrogen-like in nature) in the blood, vitamin E should be of therapeutic value.

### Toxaemia

Shute [31] divides pregnancy toxaemias into those produced by too little vitamin E and those produced by too little oestrogen. In the former type there is a raised blood pressure, oedema and albuminuria, and premature detachment of the placenta which gives rise to the name "haemorrhagic toxaemia". This type responds to treatment with vitamin E. The second is the true eclamptic type characterized by low oestrogen levels, and must on no account be treated with vitamin E, for this would still further depress the oestrogen level and cause convulsions. Instead, oestrogen therapy is required, though too much will convert the eclamptic to the haemorrhagic type.

The wheat-germ oil, according to Shute [32], should be given in high dosage and supplemented with vitamin B. An initial dose of 1 ounce of fresh oil is recommended, and then 1-2 drachms daily until the end of pregnancy. More may be needed to control the symptoms, especially towards the end of pregnancy, and in patients with hypothyroidism who tend to have high blood oestrogens. In the summer, the higher dietetic content of vitamin E decreases the requirement for wheat-germ oil.

### Menopausal Flushes

Haine [33] used wheat-germ oil in 3 or 4 cases for the alleviation of menopausal flushes and headaches, apparently with some success. The author suggests that this treatment may be given a trial, especially where an idiosyncrasy for oestrogen exists. Vitamin E for the treatment of menopausal flushes should be used only in cases accompanied by hypothyroidism; if hyperthyroidism co-exists, oestrogen should rather be given.

### Primary Sterility

Primary sterility is probably not cured by wheat-germ oil, either in men or in women, probably because deprivation of vitamin E causes irreversible testicular changes in the male, and is not necessary for conception in the female. According to Mason, some human testes show changes due to vitamin E deficiency, and Shute [32] maintains that male sterility may be cured by vitamin E.

### Lactation

Bennholdt-Thomsen [34] found that neither the fat content nor the quantity of human milk is affected by extra vitamin E. Shute claims that in a few cases wheat-germ oil in doses of 2 ounces will cure defective lactation.

### Menstrual Conditions

Amenorrhoea, dysmenorrhoea, and menorrhagic and climacteric conditions are not affected by vitamin E, with the possible exception of vaginitis and senile vulvo-vaginitis [32].

## BIBLIOGRAPHY

1. ZONDEK, H. "The Diseases of the Endocrine Gland", p. 101, Edward Arnold & Co., London, 1944.
2. KORENCHEVSKY, V., HALL, K., and CLAPHAM. *Brit. Med. J.*, **1**, 245, 1943.
3. DRILL, V. A., and TRUANT, A. P. *Endocrinol.*, **40**, 259, 1947.
4. GAEBLER, O. H., and MATHIES, J. C. *Endocrinol.*, **39**, 239, 1946.
5. BLY, C. G., HEGGENESS, F. W., and NASSET, E. S. *J. Nutr.*, **26**, 161, 1943.
6. MOORE, T. *Biochem. J.*, **31**, 155, 1937.
7. FASOLD, H., and HEIDEMANN, E. R. *Ztschr. f. d. ges. Exper. Med.*, **92**, 53, 1933.
8. MCCARRISON, R. *Ind. J. Med. Res.*, **11**, 1, 1923.
9. LOGARAS, G., and DRUMMOND, J. C. *Biochem. J.*, **32**, 964, 1938.
10. BRAZER, J. C., and CURTIS, A. C. *Arch. Int. Med.*, **65**, 90, 1940.



11. SMITH, D. C., and PERMAN, J. M. *Endocrinol.*, **27**, 110, 1940.
12. BELASCO, I. J., and MURLIN, J. R. *J. Nutr.*, **20**, 577, 1940.
13. SCHULZE, E., and HUNDHAUSEN, G. *Arch. Exper. Pathol. Pharmacol.*, **192**, 43, 1939.
14. LEONARDS, J. R., and FREE, A. H. *J. Nutr.*, **26**, 499, 1943.
15. WILLIAMS, R. K., and KENDALL, E. C. (Mayo Clinic.) *Arch. In. Med.*, **72**, 185, 1943.
16. BISKIND, M. S. *J. Clin. Endocrinol.*, **3**, 227, 1943.
17. FALK, H. C. *J. Clin. Endocrinol.*, **3**, 148, 1943.
18. BISKIND, G. R., and GLICK, D. *J. Biol. Chem.*, **110**, 583, 1935.
19. BENNETT, H. S. *Am. J. Anat.*, **67**, 151, 1940.
20. SAYERS, G. and M. A., TSANG-YING LIANG, and LONG, C. N. H. *Endocrinol.*, **38**, 1, 1946.
21. MCHENRY, E. W., and GAVIN, G. *J. Biol. Chem.*, **138**, 471, 1941.
22. GAVIN, G., and MCHENRY, E. W. *J. Biol. Chem.*, **141**, 619, 1941.
23. ROTHMAN, S. *J. Invest. Dermatol.*, **5**, 67, 1942.
24. PILLAY, A. P. *Indian. Med. Gaz.*, **75**, 91, 1940.
25. MICHAELSON, O., DIPPEL, A. L., and TODD, R. L., *Clin. Endocrinol.*, **3**, 699, 1943.
26. PHILLIPS, P. H., LANDY, H. A., BOYER, P. O., VERNER, G. M. *J. Dairy Science*, **24**, 153, 1941.
27. PHILLIPS, P. H., LANDY, H. A., HEIZER, E. E., and RUPER, S. W. *J. Dairy Science*, **23**, 873, 1941.
28. DRUMMOND, J. C., NOBLE, R. L., and WRIGHT, M. D. *J. Endocrinol.*, **1**, 275, 1939.
29. SHUTE, E. *J. Obst. & Gynec. Brit. Emp.*, **42**, 1071, 1935.
30. *Ibid.*, **43**, 74, 1936.
31. *Ibid.*, **44**, 121, 1937.
32. SHUTE, E. Soc. of Chem. Ind. "Vitamin E—A Symposium", London, 1939.
33. HAINE, A. M. *Brit. Med. J.*, **2**, 8, 1943.
34. BENNHOLDT-THOMSEN, C. *Klin. Wchnschr.*, **19**, 102, 1940.
35. ELLSWORTHY, R. *J. Clin. Invest.*, **11**, 1011, 1932.
36. ALBRIGHT, F., and SULKOWITCH, H. W. *J. Clin. Invest.*, **17**, 305, 1938.
37. SEGALOFF, A. *Endocrinol.*, **38**, 212, 1946.
38. ZONDEK, B., and FINKELSTEIN, M. *Science*, 2723, 259, 1947.
39. TOONE, E. C., and BERKLEY, J. L. *Virginia Med. Monthly*, **66**, 282, 1939.
40. SAPHIR, W. *Amer. J. Dig. Dis.*, **7**, 208, 1940.
41. CURRIE, D. W. "Vitamin E", p. 77, London Soc. Chem. Ind., 1939.
42. DRILL, V. A. *Am. J. Physiol.*, **122**, 486, 1938.
43. SEGALOFF, A. and A. *Endocrinol.*, **34**, 346, 1944.
44. ZONDEK, B., SULMAN, F., and SKLOW, J. *J. Endocrinol.*, **33**, 333, 1943.
45. BISKIND, M. S. and R. G. *Endocrinol.*, **32**, 97, 1943.
46. DRILL, V. A., and PFEIFFER, C. A. *Endocrinol.*, **38**, 300, 1946.
47. DRILL, V. A., and HAYS, H. V. *Am. J. Physiol.*, **136**, 762, 1942.
48. WARBURG, O. *Naturwissenschaft*, **22**, 441, 1934.

49. ASHWORTH, J., and SUTTON, D. C. *Arch. Int. Med.*, **69**, 15, 1942.
50. HERTZ, R. *Endocrinol.*, **37**, 1, 1945.
51. ALBRIGHT, F. "Glandular Physiology and Therapy", Am. Med. Assn., Chicago, 1942.
52. WEGELIN, G. *West. J. Surg.*, **47**, 147, 1939.
53. DAVIES, A. W., and MOORE, T. *Biochem. J.*, **28**, 288, 1934.
54. POPPER, H. *Am. J. Physiol.*, **134**, 114, 1941.
55. CANNON, M. D. *Proc. Soc. Exper. Biol. & Med.*, **44**, 129, 1940.
56. SHERWOOD, T. C. *J. Nutr.*, **11**, 593, 1936.
57. HUNDHAUSEN, G., and SCHULTZE, E. *Arch. Exp. Pathol. Pharmacol.*, **191**, 570, 1939.
58. VERZAR, F., and LASZT, F. *Arch. f. d. Ges. Physiol.*, **237**, 476, 1936.
59. GAUNT, R., LILING, M., and MUSHETT, C. W. *Endocrinol.*, **38**, 127, 1946.
60. BISKIND, G. R., and GLICK, D. *J. Biol. Chem.*, **110**, 583, 1935.
61. DEANE, H. W., and MCKIBBIN, J. M. *Endocrinol.*, **38**, 385, 1946.
62. MITCHELL, H. K., SNELL, E. E., and WILLIAMS, R. J. *J. Am. Chem. Soc.*, **63**, 2284, 1941.
63. MARTIN, G. I. *Proc. Soc. Exper. Biol. & Med.*, **51**, 353, 1942.
64. KOREF, O., and ENGEL, P. *Endocrinol.*, **38**, 133, 1946.

## DIAGNOSTIC PROCEDURES

## GONADOTROPHIN TESTS

BLOOD GONADOTROPHIN ASSAY IN NON-PREGNANT  
CONDITIONS**Fluhmann's Modified Aschheim-Zondek Test [1]**

1. 15–20 c.c. of blood from the arm vein.
2. Inject 0.5 c.c. of clear serum twice daily for 4 days into an immature white mouse 17–22 days old and weighing 6–8 grams.
3. The animal is watched over 5 days for vaginal introitus. When this has occurred vaginal smears are taken for the indication of oestrus. The animal is killed and the ovaries inspected. Mature unruptured follicles denote the presence of the follicle-stimulating factor. Haemorrhagic follicles alone or in combination with mature corpora lutea are an indication of the luteinizing factor. A positive reaction denotes that at least 50 M.U. per litre are contained in the blood.

**Blood Gonadotrophin Assay (Frank and Salmon) [2]**

1. 40 c.c. of freshly drawn blood are poured into 150 c.c. of cold acetone. A fine precipitate forms.
2. The mixture is shaken for 20 minutes, centrifugated and the supernatant acetone poured off.
3. This procedure is repeated twice with 125 c.c. of fresh cold acetone.
4. The precipitate is dried under an electric fan, powdered and extracted with 100 c.c. of water, acidifying with dilute hydrochloric acid to pH 4.8 (brom-cresol-green).
5. The mixture is stirred for 10 minutes and centrifugated. The supernatant aqueous fluid is removed and filtered through a single layer of gauze to remove suspended particles.
6. 400 c.c. of cold acetone are added to the aqueous extract and a fine buff-coloured precipitate forms. The mixture is then placed in a refrigerator overnight at 45–50° F., centrifugated and the acetone removed.
7. The precipitate is extracted with 5 c.c. of water alkalized with dilute sodium hydroxide to pH 8.5 (thymol blue), the mixture is centrifugated and the supernatant fluid removed and adjusted to pH 7 with dilute hydrochloric acid.



8. An immature female rat weighing 24–26 grams is injected with this extract in 5 doses over a period of 60 hours. At the end of 96 hours the ovaries are inspected. Mature follicles denote the presence of the follicle-stimulating factor, and haemorrhagic follicles and mature corpora lutea the presence of the luteinizing factor, the blood contents of both being at least 25 R.U. per litre.

#### URINE GONADOTROPHIC ASSAY IN NON-PREGNANT CONDITIONS

##### **Urine Gonadotrophin Assay (Frank and co-workers) [3]**

1. 400 c.c. fresh or stored ( $4.5^{\circ}$  C.) urine are acidified with glacial acetic acid to pH 3.5 (brom-cresol-green).
2. 1,600 c.c. of 95 per cent ethyl alcohol are added to the urine and the mixture placed into a refrigerator at  $4.5^{\circ}$  C. from 6 hours to overnight.
3. The supernatant liquid is removed and the precipitate centrifugated in a 250-c.c. centrifuge tube.
4. The precipitate is washed three times with 75 c.c. of ether for each washing to remove the oestrogen.
5. The precipitate is dried under a fan and transferred quantitatively to a 15-c.c. centrifuge tube, using no more than 6 c.c. of distilled water for the transfer.
6. The preparation is triturated thoroughly with a glass rod and then centrifugated for 10 minutes. The supernatant liquid is poured into a graduated centrifuge tube and distilled water added to the 6-c.c. mark.
7. Immature female rats weighing 30 grams are then injected with this solution without further pH adjustment. Mature follicles, haemorrhagic follicles and mature corpora lutea indicate respectively the presence of follicle-stimulating and luteinizing principle in the urine.

##### **Urine Gonadotrophin Assay (Varney and Koch) [4]**

1. The urine is chilled and adjusted to a pH 4.8–5.2 with acetic acid, and 4 volumes of ethanol are then added.
2. A crude alcohol precipitate is thus formed and collected by settling and centrifugating in the freezing-room. This precipitate can be injected without further treatment when urine of high gonadotrophic content is assayed. With less

active urines the authors recommend removal of the toxic substances.

3. The crude alcohol precipitate is extracted with 50 per cent ethanol and re-precipitated from the solution by adding 4 volumes of alcohol.
4. 4 or more immature female white mice weighing 6–8 grams are used for each dosage level. Levels injected are calculated so that a positive response in each successive dosage group of animals corresponds to 10, 20 and 30 units per 24-hour-sample of urine from normal males and females. The material to be assayed is dissolved or suspended in 3 c.c. of saline and 0.5 c.c. is injected subcutaneously twice daily for 3 days. The animals are killed on the morning of the fifth day and their uteri removed, freed of connective tissue and fluid and weighed on a torsion balance. A mouse uterine unit is defined as the amount of activity necessary to cause a 100 per cent increase in uterine weight above that of the controls.

#### URINE FOLLICLE-STIMULATING HORMONE ASSAY KLINEFELTER AND OTHERS [5]

Testing for small amounts of gonadotrophin requires greater concentration of the hormone, which has the disadvantage of producing toxic substances which kill the mice.

Unconcentrated urine can be tested for only 40 m.u. per 100 c.c., since a mouse can tolerate only 2.5 c.c. of fluid (0.5 c.c. at each of the injections). By precipitating the hormone with ethyl alcohol it can be concentrated 4 times and hence one can test for 10 m.u. per 100 c.c. Further concentration with alcohol produces toxic substances. Heller and Heller [146], were the first to introduce a dialysing procedure which eliminates toxic substances, making it possible to concentrate the hormones to any degree, and thus assay specimens for normal and subnormal amounts of hormone.

Two modified procedures are described.

#### **The Non-dialysis Method**

##### *Preparation*

1. Take a 90-minute aliquot of fresh overnight urine; make acid to litmus and filter if cloudy.
2. Precipitate with 8 volumes of cold (ice-box) 95 per cent ethyl alcohol in a 1- or 2-litre Erlenmeyer flask. Let stand in ice-box overnight or until the supernatant fluid is clear.

3. Siphon off most of supernatant fluid and collect precipitate in a 50-c.c. centrifuge tube, using some of the supernatant fluid for transfer.
4. Wash the precipitate twice with 15 c.c. of absolute alcohol and once with 15 c.c. of absolute ether; centrifuge each washing until the supernatant fluid is clear. The second alcohol washing may remain cloudy after considerable centrifuging; a few c.c. of absolute ether and an increased speed of the centrifuge hasten the complete clearing. The same glass stirring-rod should be used throughout this and subsequent procedures.
5. Dry the precipitate in a vacuum desiccator overnight. The dried precipitate is stable and can be stored at room temperature until assayed.
6. On the day before assay is begun, add 15 c.c. of distilled water to dry precipitate, mix thoroughly and allow it to stand in ice-box overnight before dilutions are made.

### Assay

1. The criterion of a positive test is based on the weight of the mouse uterus. Two immature (*circa* 21 days) female white mice weighing 6–10 grams are used at each dilution level. They are injected subcutaneously 5 times over a period of 3 days (twice the first day, twice the second and once the third) with 0.5 c.c., each mouse receiving a total of 2.5 c.c. The mice are killed with coal-gas 72 hours after the first injection. The uterus is considered enlarged if it weighs 7 mg. or more after the fluid has been expressed by pressing between layers of filter-paper. Uteri are weighed only when there is questionable enlargement.
2. Calculations: The final solution used for assay (15 c.c.) represents the original 90-minute fraction of urine. If 2.5 c.c. gives a positive result, the 90-minute fraction contains at least 6 mouse units of follicle-stimulating hormone, or 1 unit for 15 minutes or 96 units per 24 hours.
3. Dilutions for testing various levels of the hormone are made as follows and kept in the ice-box:

M.U.	24-hr. c.c. of Solution	c.c. Distilled Water
96	6.0	0.0
192	3.0	3.0
288	2.0	4.0
384	1.5	4.5
480	1.2	4.8



## Dialysis Method

### *Preparations*

1. Take one or more fresh, overnight urine specimens; make acid to litmus and filter if cloudy. The time of collection represented must be at least 8 hours if the test is to be made for 6.6 m.u. per 24 hours; 16 hours, if test is for 6.6, 13.2, 26.4, and 52.8 m.u. per 24 hours.
2. Add 1 gram of sodium chloride for each 100 c.c. of urine. Precipitate with 4 volumes of 95 per cent alcohol in a large Erlenmeyer flask. Let stand in ice-box overnight or until supernatant fluid is clear.
3. Siphon off most of the supernatant fluid and collect the precipitate in a 250-c.c. centrifuge bottle, using some of the supernatant fluid.
4. Wash the precipitate once with 50–60 c.c. of absolute ether. Use the same glass stirring-rod throughout this and subsequent procedures. Dry the precipitate overnight in vacuum desiccator.
5. Extract the precipitate 3 times with 10–15 c.c. of distilled water each time. After thoroughly breaking up and mixing the precipitate, allow each extraction solution to stand for 30 minutes. Centrifuge each extraction solution for 3–5 minutes and pool them in a small Erlenmeyer flask.
6. Transfer this solution (30–45 c.c.) to a cellophane bag  $1\frac{1}{8}$  inches in diameter when inflated; use 2–3 c.c. of distilled water to wash out the flask. Dialyse against running cold tap-water for 4 hours.
7. Re-precipitate the dialysate, after adding 0.1 gram of sodium chloride, with 4 volumes of 95 per cent. alcohol, and let stand in ice-box overnight or until the supernatant fluid is clear.
8. Siphon off most of the supernatant fluid and collect precipitate in a 50 c.c. centrifuge tube, using some of the supernatant fluid.
9. Wash the precipitate once with 15–20 c.c. of absolute ether, and dry overnight in a vacuum desiccator. This precipitate is stable and can be stored at room temperature until assayed.
10. On the day before the assay is begun add 5.5 c.c. of distilled water to the dry precipitate of an 8-hour specimen, or 11 c.c. of distilled water to the dry precipitate of a 16-hour speci-

men, mix thoroughly and allow to stand in the ice-box overnight.

### Assay

1. The assay is made in the same way as that outlined for the non-dialysis method.
2. Calculations: The final solutions (5.5 or 11 c.c.) represent the whole 8- or 16-hour urine collections, respectively. If 2.5 c.c. give a positive result, the whole specimens contain at least 2.2 or 4.4 m.u. of follicle-stimulating hormone respectively. If an 8-hour collection contains at least 2.2 m.u., a 24-hour collection will contain at least 6.6 m.u. ( $2.2 \times 24/8$ ). If a 16-hour collection contains at least 4.4 m.u., a 24-hour collection will contain at least 6.6 m.u. ( $4.4 \times 24/16$ ).
3. Dilutions: To test for 13.2, 26.4, 52.8 m.u. or higher amounts of follicle-stimulating hormone, the necessary dilutions are made with distilled water. It is important that each mouse should receive the same amount of fluid (2.5 c.c.) and that solutions be kept in the ice-box between injections.

### Ultrafiltration Method [182, 183]

1. A 12-hour aliquot of urine is placed in a pressure filter and forced through a collodion membrane.
2. The membrane along with the protein hormone residue which has not passed through the pores of the membrane owing to the large size of the protein molecule is removed from the filter and placed in alcohol-ether solution in a centrifuge tube.
3. The solution is centrifugated, the supernatant fluid poured off and the residue repeatedly (5 times) washed with alcohol-ether.
4. After the last centrifugation, the residue is allowed to dry in the centrifuge tube.
5. At the time of assay the dry precipitate is extracted with 6 c.c. of water.
6. The extract is injected into 22-24 female rats once every 12 hours for 6 injections.
7. Twenty-four hours after the last injection the rats are sacrificed and the uterine and ovarian weights used as the assay end-point.

At lower dose levels (6-hour urine aliquot of a normal person) uterine weight increase occurs without ovarian weight increase. At

higher dose levels, ovarian weight increase, along with maximal uterine weight increase, is observed.

This method takes 5 hours while 44-92 hours are required for the alcohol-precipitation method.

It appears that with the membrane technique the recovery is less complete than with the alcohol-precipitation method.

#### SOUTH AFRICAN MALE FROG TEST FOR GONADOTROPHINS [184]

A new laboratory animal, the male South African clawed frog (*Xenopus laevis*), is now available for endocrinological assay, particularly in the field of sex hormones. This animal is many times more sensitive to pituitary and chorionic gonadotrophins than the standard laboratory animals now used for these assays, thus permitting the detection of hormone levels too small to be determined by methods heretofore available. Moreover, it possesses the significant advantage of enabling an assay to be carried out within a few hours instead of requiring, as with other animals, one to several days. The obvious applicability of this new test to the field of pregnancy diagnosis and other endocrinological studies is now under investigation.

Using gonadotrophic hormones, positive reactions develop within  $1\frac{1}{2}$  to 2 hours after the administration of the hormone. The onset of the appearance of spermatozoa may be quite abrupt, but in general the reaction develops progressively, reaching a peak within 2-3 hours after administration of the hormone. In instances of "weak positives", the spermatozoa may begin to disappear within 3-4 hours after their initial appearance. However, even in the weakest responses to the hormones, spermatozoa can be observed for periods of at least 2-3 hours, and hence periodic examinations spaced 1-1 $\frac{1}{2}$  hours apart can be relied upon to detect the presence of a positive reaction. Occasionally strongly positive tests will result in continuous extrusion of spermatozoa for 12-20 hours. If examinations are repeatedly negative for 4-5 hours after the hormone injection has been given, it may safely be assumed that the result is negative. After the completion of a positive test, the animal is given a rest for a 7-day period, at which time it is again ready for use. Somewhat shorter rest periods of 3-4 days suffice following a negative test. Like the female, these male frogs may be used repeatedly.

Using these methods the following comparative results were obtained: The male frog was found to be 64 times as sensitive to pituitary gonadotrophic hormones as the female, and 128 times as sensitive as



the rat. Furthermore, the male proved to be twice as sensitive as the rat to chorionic gonadotrophic hormone and 10 times as sensitive as the female frog.

These relationships can be summarized as follows: For pituitary gonadotrophins 1 rat unit is equivalent to 2 female frog units and 128 male frog units; for chorionic gonadotrophins 1 rat unit is equivalent to one-fifth of a female frog unit and 2 male frog units. It should be noted that the sensitivities of the male and female frogs compared to each other and to the rat are distinctly different for gonadotrophins of pituitary and chorionic origin.

### THE HYPERAEMIA TEST FOR CHORIONIC GONADOTROPHIN [185]

The hyperaemia reaction is elicited by prolان B (LH) in the ovary of infantile rats only if prolان A (FSH) is present. Urine which contains only prolان A does not elicit the hyperaemia response [15].

A hyperaemia unit (1 HU) is the minimum amount of gonadotrophic hormone which, when given by single injection, elicits in the rat ovary a red colour similar in tone to that of spleen, kidney or liver. The hyperaemia reaction is unequivocally demonstrable 10–24 hours after the injection. Two to ten hours after the injection the ovary reaction is pink rather than red and can be definitely detected only by an experienced observer through reference to the swelling with which hyperaemia is associated. The difficulty of making the reading at this time interval is evident from the work of Farris [13], who obtained paradoxical results using the 2-hour test. If the reaction is read at 10 hours or later, the positive colour reaction is unequivocally demonstrable where a dosage greater than the threshold dose has been given. The hyperaemia unit is less liable to variation than the oestrus and luteinizing units. A hyperaemia unit (1 HU) is therefore defined as the minimum amount of hormone which elicits a positive reaction in both ovaries of a rat in each animal of a group of 4.

Comparison of the values of HU and RU\* shows that 20 RU are necessary to evoke a positive hyperaemia reaction in the ovary 2 hours after the injection. The effective dose is found to decrease as the time

\* An oestrus unit (RU) is the minimum amount of gonadotrophic hormone which produces a positive vaginal-smear reaction (keratinization) 72–96 hours after injection of a single dose into a 3–4-weeks-old rat weighing 25–30 grams. Vaginal smears are made at intervals of 72, 84 and 96 hours after the injection. The unit is defined as the minimum amount of hormone which elicits a positive reaction in 50 per cent of the injected animals.

lapse between injection and reading is increased. It may be noted that when the time span is 6 hours, 14 RU are still necessary, but that at 8 hours 1 RU suffices. Before 8 hours the ovarian reddening is less than maximal. After 10 hours the characteristic red reaction is produced. At 10 hours the effective hyperaemia dose (HU) is actually smaller than 1 RU; at this time interval, 0.5 RU suffices to produce a definite hyperaemia effect. At periods greater than 10 hours, the reaction becomes increasingly less sensitive, the number of RU required to produce a positive response increasing between 12 and 24 hours from 2 RU to 5 RU.

### **Normal Values for Gonadotrophin Excretion**

#### *Males*

Follicle-stimulating hormone excreted in the urine:

More than 6.6 m.u. per 24 hours [5].

4-19 m.u. daily [84].

Up to 50 m.u. per litre [85].

At least 25 m.u. per litre [86].

6-36 rabbit units [87].

#### *Females* (regularly menstruating)

Follicle-stimulating hormone:

More than 6.6 and less than 53 m.u. per 24 hours' urine [5].

At the mid-cycle a peak of gonadotrophin excretion consisting of both fractions occurs. Different values are reported by various investigators.

25 rat units per litre of blood [88].

2-25 rat units per litre of urine [89].

5-15 rat units per litre of urine [90].

16 rat units per 24 hours [91].

Some observers report a second peak of gonadotrophin excretion before menstruation.

### **Pregnancy Values for Gonadotrophin Excretion**

Excretion of gonadotrophic hormone, predominantly the luteinizing fraction, begins within a few days after the first missed period, progressively increasing in amount until a peak is reached during the first third of gestation, after which the level gradually drops. Blood values roughly correspond to those in the urine [92].

The following excretion rates have been observed:

### *Blood*

Between the 30th and 46th day: 100,000–500,000 rat units per litre [93].

Between the 5th and 6th week: 1,600 m.u. per 100 c.c. of blood.

Between the 8th and 10th week: 3,000 m.u. per 100 c.c. of blood.

Between the 20th and 24th week: 300 m.u. per 100 c.c. of blood [94].

Between the 50th and 65th day: 150,000 I.U. per litre.

At the 100th day: 10,000–12,000 I.U. per litre.

From the 130th day until the end of pregnancy: 5,000–6,000 I.U. per litre.

There is some indication of a slight secondary rise between the 200th and 230th day [173].

### *Urine*

Between the 52nd and 64th day: 133,000–40,000 rat units per litre.

At the 67th day: 40,000 rat units per litre.

After the 120th day: 1,500–5,000 rat units per litre [95].

## **Menopausal Values for Gonadotrophin Excretion**

Follicle-stimulating hormone excreted in the blood:

50 m.u. per litre [96].

Follicle-stimulating hormone excreted in the urine:

110–400 m.u. per litre [97].

100–550 m.u. per 24 hours [98].

25–66 rat units per litre [90].

75 rat units per 24 hours [84].

## **PREGNANCY TESTS**

### **URINE GONADOTROPHIN ASSAY**

#### **Modified Aschheim-Zondek Test**

For the original Aschheim-Zondek reactions [6], the following modification has been suggested by Zondek.

1. 66 c.c. of morning urine are slightly acidified with a few drops of 10 per cent acetic acid and then filtered.
2. 240 c.c. of 96 per cent ethyl alcohol are added and shaken for 5 minutes when a yellow-white precipitate forms.



3. After 30 minutes this is centrifugated, the sludge taken up and thrown down to 30-50 c.c. of ether, and shaken for 3 minutes.
4. The ether is removed and the residue taken up in 11 c.c. of distilled water; it is then shaken for 5 minutes and centrifugated. The supernatant fluid is preserved for injections. It contains the active principle and represents a sixfold concentration of the urine. Each of 4 immature female mice are injected with 0.4 c.c. of this substance 4 times on the first day and twice on the second day.
5. 51-57 hours after the first injection the ovaries are inspected and the presence of haemorrhagic follicles denotes a positive reaction.

### **Rapid Ovulation Test [186]**

This is performed on mature mice in dioestrus and requires only 18-24 hours.

1. A first morning specimen of urine is used.
2. A single injection of 0.1-1.0 c.c. of untreated urine is made subcutaneously into mature albino mice weighing at least 20 grams and having an abundance of leucocytes typical of dioestrus.
3. Autopsy is performed 18-24 hours later. Ovulation is manifest by the distension of the ampulla of the oviduct with fluid and the egg mass and can be seen with a dissecting microscope.

The minimal ovulating dose of urine containing 10,000 mouse-ovulating units per litre is 0.1 c.c. The chorionic gonadotrophin content of urines from pregnancies of 6-8 weeks contains from 10,000 to 80,000 mouse-ovulating units per litre.

### **Friedman Test [7]**

1. A first morning specimen of urine is used.
2. The experimental animal is an adult female rabbit in oestrus, weighing no less than 4 pounds, which has been isolated for 3 weeks before being used.
3. 4 c.c. of urine is injected 3 times on each of 2 successive days into the marginal vein of the rabbit's ear.
4. At the end of 48 hours after the first injection laparotomy is performed, or the animal is killed and the ovaries examined. Fresh corpora lutea or large corpora haemorrhagica indicate a positive reaction.

Modifications of the Friedman test turn on whether the animal selected is immature or post-partum. A single injection of 10 c.c. of urine [8], or followed 12 hours later by a second injection of 5 c.c. [9], have been used. Inspection of the ovaries 24 hours after the first injection is less reliable than after 48 hours.

### **Six-hour Pregnancy Test [10]**

1. The first morning specimen of urine is used.
2. The experimental animals are two 4-week-old female rats, weighing about 30 grams, in whom neither vaginal introitus nor corpus luteum formation has occurred.
3. 2 c.c. of urine are injected subcutaneously into each rat.
4. After 6 hours the animals are asphyxiated with coal-gas, and a diagnosis of pregnancy can be made if ovarian hyperaemia is manifested by a reddening of the ovary, which is visible in good light. Usually the uterus, too, is oedematous and red. In negative cases the ovaries are small and white and the uterus has a white thread-like structure.

In a modification of this test the injection of 5 c.c. of urine instead of 2 c.c. is recommended, and the use of rats of any age and weight in metoestrus or dioestrus is described as satisfactory.

### **Two-hour Pregnancy Test [11]**

1. The morning specimen of urine is used.
2. Immature rats of 21–55 days of age are selected.
3. 1 c.c. of urine is injected intraperitoneally into each of the right and left lower abdominal quadrants.
4. The animals are killed with ether 2 hours after the injection. Hyperaemia as indicated by the reddish appearance of the ovary and the ovarian capsule is considered a positive reaction.

It appears that the 2- and 6-hour rat tests are not reliable. Although the results in pregnancy agree in all cases with those of the Friedman test [12, 13] they have proved positive in conditions other than pregnancy, as for example, in women undergoing menopausal changes, in normal women for 3 successive days during the mid-interval of the cycle with a range from the twelfth to the eighteenth day, and in men and women after coitus [13, 14]. Bunde [187] found that the test was correct in 84.5 per cent of cases when two rats were used and in 90.5 per cent when the number was increased to three.

All errors were failures to get a positive response from urines of pregnant women.

### **Twenty-four-hour Pregnancy Test [15]**

Zondek and co-workers [15] have recently described a modification of the 2- and 6-hour rat tests.

1. The morning specimen of urine is used.
2. Three infantile female rats 3-5 weeks old, weighing 20-5 grams, are selected.
3. 4 c.c. of urine are injected subcutaneously into the neck of each of the animals, in 2 injections of 2 c.c. each, at an interval of 1 hour.
4. One rat is killed after 6 hours and the other two are killed after 24 hours.
5. The reaction is positive if hyperaemia appears in at least one ovary of two different rats. As a rule all the ovaries react similarly.

The authors maintain that this test is more reliable than the 2- and 6-hour tests and ascribe to the 2-hour test an accuracy of 69 per cent, to the 6-hour test 92 per cent, and to the 24-hour test 100 per cent. There may be, however, occasional positive results with the 24-hour test with non-pregnant patients with chorionepithelioma and hydatidiform mole.

### **The Frog (*Xenopus laevis*) Test [14, 16]**

1. 80 c.c. from the morning specimen are taken and 2 volumes of acetone are added. The mixture is stirred thoroughly and allowed to stand for about 15 minutes until the hormones and proteins precipitate and settle at the bottom. The supernatant fluid is decanted and saved for redistillation.
2. The precipitate is dried with an electric fan and 2 c.c. of distilled water are added and the mixture is thoroughly stirred. It is then centrifugated in a small tube to bring down insoluble proteins and other material leaving the gonadotrophin in solution.
3. Immediately before injection the supernatant fluid is removed and adjusted with 10 per cent sulphosalicylic acid to pH 5.5. For each injection 1 c.c. of concentrate is used (being equivalent to 40 c.c. of whole urine).
4. The frogs are kept in groups of eight in 3 12-gallon aquariums. In one tank the frogs recuperate for 4 weeks after having



extruded eggs in a positive reaction. In the second tank frogs rest for 1 week after having shown a negative reaction, and the third is used for animals ready for the test. The tanks are kept with water at 70° F. and at a level of 3 inches from the bottom and replaced with fresh water of the same temperature before and 24 hours after each feeding. The animals are fed with small strips of beef heart, calves' liver and garden worms twice a week.

5. For the test a 2-gallon fish tank is used containing a half-inch wire mesh placed about 1 inch from the bottom.
6. 1 c.c. of extract is injected in 1 or 2 frogs into the dorsal lymph sac.
7. The animals are observed at intervals beginning 4 hours after the injection. Extrusion of eggs taking place between 4 and 12 hours after the injection signifies a positive reaction.

Each frog can be used 24 times [16], and a high degree of accuracy (98-100 per cent) is claimed [16, 17, 18].

Only animals which have previously ovulated after injection of gonadotrophic extract should be used. Frogs in use for the first time may be primed 3 weeks beforehand by the injection of 100 I.U. chorionic gonadotrophin [16].

A modification of the test, both with respect to the concentration of urine and to performance, is described by Landgrebe and others [16]. Using the Scott's method [19], each frog is injected with 2½ c.c. of urine extract into the dorsal lymph sac.

### Scott's Method of Urine Concentration

1. To 100 c.c. of urine in a 250 c.c. graduated cylinder add 100 c.c. of water.
2. Add 1 c.c. of brom-phenol blue indicator and adjust to pH4.0 by addition of 20 per cent hydrochloric acid, adding about 0.25 c.c. of acid at a time, with constant shaking until the colour changes from deep blue to a point where the blue almost disappears.
3. 10 c.c. of well-shaken suspension of kaolin (20 per cent aqueous suspension B.D.H. kaolin washed with acid) is added and the urine thoroughly mixed by inversion several times. Allow the kaolin to settle to a layer below the 30 c.c. mark.
4. Pour off the supernatant fluid and centrifuge the kaolin suspension.
5. Decant the supernatant fluid and thoroughly stir the kaolin

with 10 c.c. of N/10 sodium hydroxide. Stand the mixture for a few minutes and then centrifuge.

6. Decant the supernatant fluid into another tube, neutralize it by the addition of about 0.75 c.c. 10 per cent hydrochloric acid and add about 1 gram of solid glucose.
7. Dissolve the glucose, and the extract is ready for immediate injection or is stable for several weeks if kept in a refrigerator.

Only concentrated urine should be used, since untreated urine gives an accuracy of less than 50 per cent [20].

Menopausal urine, which usually gives a positive Aschheim-Zondek or Friedman reaction, invariably fails, even when concentrated, to induce egg-extrusion in the frog [21].

According to Foote [188] pregnancy cannot be diagnosed by the Hogben frog test with accuracy before the fortieth day of gestation. Following this period the expected accuracy approaches 96 per cent. Approximately two-thirds of threatened or incomplete abortions will give positive Hogben tests. Cases of corpus luteum cysts, suspected of being ectopic pregnancies, give negative reactions. These results are comparable with those obtained with the Friedman test and show the Hogben test to be a satisfactory one for clinical use. The most important clinical advantage of the Hogben over the Friedman test, apart from technical superiority in the laboratory, is that it gives no false positive results.

### Three-hour Colour Test [22]

This test is based on the colour reaction of pregnanediol.

#### *A. Hydrolysis and Extraction of Pregnanediol*

1. 100 c.c. urine (first morning specimen), 50 c.c. toluene (C.P.), 10 c.c. concentrated hydrochloric acid, and 2 glass beads are added to a 500-c.c. flat-bottomed Florence flask.
2. The flask is connected via one-holed cork stopper to a vertical Liebig condenser (water-cooled, 400–500 cm. jacket length) and the mixture is boiled vigorously over an electric hot-plate for 15 minutes.
3. The flask and its contents are brought to room temperature by cooling under the water-tap.
4. The mixture is transferred to a 500-c.c. separating funnel, and the lower layer (urine) is drawn off.
5. The toluene layer and the toluene-water emulsion are washed, twice with 15-c.c. portions of N/10 sodium hydroxide, and then twice with 15-c.c. portions of distilled water.

### *B. Precipitation of Impurities*

1. The washed toluene and toluene-water emulsion (A-5) are transferred to a 125-c.c. Erlenmeyer flask with 2 glass beads.
2. The mixture is boiled over an electric hot-plate (in the hood).
3. When the water has evaporated and the toluene mixture is boiling smoothly, 10 c.c. of 2 per cent sodium hydroxide in absolute methanol is added.
4. The mixture is evaporated until one-half of the original toluene volume is reached.
5. The toluene mixture is then filtered, while hot, through a fritted glass filter (medium porosity Pyrex), with mild suction. (If the filtrate has an orange, pink or brown tinge, steps B-3, B-4, B-5 must be repeated until the filtrate is yellow or yellow-green.)
6. The precipitate (B-5) is washed with 15 c.c. hot toluene.
7. The combined filtrates (B-5 and B-6) are then evaporated to dryness over the hot-plate (in the hood), a gentle airstream being used to drive off the last traces of toluene. This avoids charring of the residue.

### *C. Precipitation of Pregnanediol*

1. 5 c.c. acetone is added to the residue (B-7) and the mixture is warmed over a hot-plate until the solution is complete.
2. 20 c.c. of N/10 sodium hydroxide is added slowly while the mixture is still on the hot-plate.
3. The flask is then placed in an ice-water bath for 30 minutes or in a refrigerator (5° C.) for 1 hour.

### *D. Isolation of Pregnanediol*

1. The mixture (C-3) is filtered through a fritted glass filter (medium porosity Pyrex) with mild suction.
2. The precipitate (D-1) is washed with 15 c.c. distilled water.
3. The receiving flask is changed, and 10 c.c. of hot absolute alcohol is passed through the fritted glass filter to dissolve the precipitate.
4. The alcohol filtrate (D-3) is evaporated to dryness over an electric hot-plate (in the hood).

### *E. Colour Development*

1. 10 c.c. concentrated sulphuric acid is added to the residue (D-4) and the colour is observed in a test tube when solution



is complete. Colourless to light yellow solution is read as negative. Orange to deep orange-brown is read as positive.

Since the first description of this test the standard for a "positive" reaction has been altered to a deep yellow colour which avoids "false negative" results and does not increase the incidence of "false positives" [189].

Pregnancy was diagnosed with this test as early as 6 days after a missed period and 21 days after artificial insemination. It appears more accurate than the Friedman test, and has the added advantages that it is more rapid, more specific and more economical, and does not require animals with attendant housing and feeding facilities.

Conditions which are associated with the excretion of luteinizing hormone, such as hydatidiform mole and testicular tumours, give a positive to the Friedman technique. Pregnanediol determinations in such cases, however, yield negative results.

The chemical test plus the Friedman test are good indicators in cases of threatened abortion. The chemical test is negative in threatened abortion due to the production of little or no progesterone. By contrast, the Friedman test depends upon the anterior-pituitary-like substance elaborated by chorionic villi. A positive chemical test will be obtained in the presence of a corpus-luteum cyst, but this condition is comparatively uncommon [190].

Morrow and Benua [191] found the Guterman colour test positive in a large percentage of non-pregnant women during the luteal phase of the menstrual cycle, in amenorrhoea due to a functioning corpus luteum, and when large amounts of 17-ketosteroids are present in the urine. In one case of an apparently normal pregnancy of 7 weeks' duration, the Guterman test gave a negative result. According to Guterman [192] the explanation of these contradictory results was that the method was not followed in important details, and that the colorimeter assay was done at a wave-length which might include substances other than pregnanediol.

### **Prostigmine Pregnancy Test**

Prostigmine methylsulphate (1 : 2000) 1 c.c., injected intramuscularly on three successive days, usually produces bleeding in the non-pregnant woman within 72 hours of the last injection. If no bleeding occurs within this time a tentative diagnosis of pregnancy may be made [23]. Cases of amenorrhoea due to endocrine disorders or organic diseases, however, do not respond with bleeding [23], and pregnant women may bleed after prostigmine administration [24].

Grossman [25], on the other hand, recently reported 100 per cent accuracy with this test.

### **Histidine Pregnancy Test**

The Kapeller-Adler test for the excretion rate of histidine has been suggested as a rapid method for the diagnosis of pregnancy [26, 27]. An accuracy of 91 per cent in pregnancy and of 95 per cent in non-pregnant women of childbearing age is reported, with no errors in menopausal women and males [27].

### **ESTIMATION OF OESTROGENS**

Most biological tests for the oestrogen content of blood and urine are based on the assay method of Allen and Doisy [28], whereby oestrus is induced in the ovariectomized rat or mouse. Another method of assay turns on the production of increased weight of the uterus in the immature rat [29].

#### **Blood Oestrogen [30]**

1. The bottom of a petri dish is covered evenly with 30 grams of finely powdered anhydrous sodium sulphate.
2. 50 c.c. of venous blood are drawn and transferred at once to the petri dish.
3. The blood and powder are stirred until thoroughly dry, and the small lumps formed are triturated in a mortar.
4. The powder obtained is extracted twice, each time with 200 c.c. of 95 per cent alcohol. The alcohol fractions are combined, and then evaporated to dryness on a water bath, and 5 c.c. of oil are added to the residuum.
6. For assay the Allen and Doisy method is used. The oil solution is injected in 3 equal doses at 3-4 hour intervals to an adult female white mouse spayed 2 weeks previously. Twenty-four hours after the last injection, a vaginal smear is taken and 3 more are made at 12-hour intervals, with a final one on the third morning after the last injection. A positive oestrogenic smear indicates that the blood contains at least 25 mouse units per litre.

In modification of this test [31] centrifugated clear blood-serum is injected to a total of 4.5 c.c. 3 times daily on 3 consecutive days into 2-3 spayed adult female mice. On the fourth day the animals are killed and the vaginas dissected, fixed in formalin and mounted

in paraffin, and transverse sections are made at different levels and stained with haematoxylineosin. As few as 3 mouse units of oestrogen can be assayed by the histological changes in the vagina.

### Assay of Oestrogens in Minute Amounts

A new test for blood oestrogen of extreme sensitivity has recently been described by Hartman and Littrell [174]. It turns on the well-known fact that the vagina of the rat remains closed until sexual maturity, but opens earlier in response to the administration of oestrogens.

The procedure is extremely simple. The sample, 0.01 or 0.02 c.c. in volume, is injected subcutaneously in the 21-day rat near the region of the future vaginal orifice and observations are made once daily. The first indication of a positive reaction consists of a crescent-shaped transverse dimpling of the skin at the developing vaginal orifice. Openings appear at various points in the crescent and fluid oozes from them on gentle pressure.

On the average it takes 4 or 5 days for the vaginae of two out of three animals to open; but easily recognizable changes usually appear within 24 hours. If large doses are used, for example 0.005 mg. of oestradiol dipropionate in 0.02 c.c. of oil, the vagina may open within 24 hours and definite changes, easily recognizable at a glance, have been noted within 17 hours.

The test is claimed to be extremely sensitive to blood oestrogens, for as little as 0.02 c.c. of untreated finger blood from women was always positive, while that from men was always negative.

For most of the experiments, 21-day-old female rats have been used; but in one experiment 6 females of one litter of 16-day-old rats were used with such success that it seems possible that the 16-day-old rat may become the animal of choice. The young were injected and returned to their mother. Within 4 days all the injected females had open vaginae: the 2 which had received 0.005 mg. of oestradiol in 0.02 c.c. of oil and the 3 which had received 0.02 c.c. of mid-cycle female blood. The dimpling was observable within 30 hours. The one control showed no change.

How delicate this test really is, in terms of blood oestrogen, is seen in the following calculation. Taking the recent figures of Markee and Berg [175] as a basis, the mid-cycle titre of oestrogen in the blood was found to be roughly 0.005 mg. per litre or 0.000005 mg. per c.c. A positive reaction, involving anatomical changes, is thus attained with five one-hundred-millionths of a milligram of oestrogen—which, however, still contains some billions of molecules.

The test, it is to be noted, costs nothing, for the test animals are



not sacrificed, remaining perfectly normal members of a colony. Furthermore, the time required to make and read the test is almost negligible.

A modification of this test [193] consists in using, instead of rats, weanling guinea pigs weighing about 200 grams or spayed adult guinea pigs. The latter have to be primed a week before use and can be used repeatedly since the membrane regenerates after 4 days. The total dosage is administered in two injections, one-half on either side of the closure membrane. The needle is inserted at the tip of each horn of the crescentic depression extending across the perineum, and is directed towards the midline underneath the horn of the crescent. Since it is important that the injection be made with precision, a second operator is essential in order thoroughly to immobilize the animal. The end-point of the test is the opening of the vaginal membrane which occurs in the following stages: line formation, dimpling, pinpoint formation, slit formation, complete opening.

Lloyd and co-workers [194] repeated this method in rats aged from 16 to 24 days and found that the inconstancy of results and lack of sensitivity of this method preclude its use for quantitative assay. It is obvious that further studies are required for its evaluation.

The great sensitivity of the perineal region to oestrogenic substances (in contrast to the reaction of the body wall) was described by Espinasse [176]. When arachis oil in doses of from 0.0025 c.c. to 0.005 c.c. was injected into the perineum, proliferation of the vaginal epithelium, resembling that preceding oestrus, followed in 24-48 hours. The increase in the height of the epithelial cells was only slightly noticeable when arachis oil was injected into the body wall. Thus, new difficulties arise in the interpretation of oestrogen assays, particularly when they involve injection of oil solutions into the perineum.

#### URINE OESTROGENS

Two kinds of tests are described, the one for the estimation of the free active fraction only, carried out with untreated urine, and the other to determine the total oestrogen content of the urine by converting the inactive conjugated form through acid hydrolysis to the active free form.

#### **Total Urine Oestrogens [32]**

1. For each litre of a 24-hour specimen of urine, 200-800 c.c. are measured in to an Erlenmeyer flask, the amount depending on the probable content of oestrogen.

2. 15 volumes per cent of concentrated hydrochloric acid are added and the mixture vigorously boiled for 10 minutes.
3. The material is transferred to a large extraction flask, both flasks being filled to the neck with benzene. Extraction is carried out for 24 hours.
4. The large flask containing the extracted urine and benzene is disconnected, emptied and put back in place. The benzene in the smaller flask is then distilled into it.
5. 6 c.c. of olive oil are added to the concentrated hormone and 0.75 c.c. injected into each of 2 castrated adult female rats.
6. A positive oestrogenic smear indicates 10–40 rat units of total oestrin per 24 hours, depending on the amount of urine (200–800 c.c.) extracted. If 200–800 give a negative reaction, progressively larger amounts have to be used.
7. Calculation:  $(24\text{-hour volume} \div \text{c.c. extracted}) \times \frac{6}{0.75} =$   
rat units of oestrogen in 24-hour volume of urine.

### **Estimation of "Free" Oestrogen in the Urine when present in Increased Amounts [33]**

1. 4 mature castrated female mice each weighing 20–25 grams are selected.
2. 12 c.c. of slightly acidified filtered morning urine are injected subcutaneously into each rat in 6 divided doses over a period of 2 days.
3. On the third and fourth day vaginal smears are taken 3 times daily in order to detect the occurrence of oestrus.
4. The test is positive if, on the fourth day after the first injection, the vaginal smear shows a preponderance of non-nucleated epithelial cells and the absence of leucocytes and mucus.

A number of other tests have been described for the estimation of total urine oestrogen [34, 35, 36, 37, 38] and for the separate determination of oestrone, oestriol and oestradiol [39, 40].

### **Estimation of Oestrogens by Fluorimetry**

The sample to be tested must be well purified, preferably as far as the so-called phenolic fraction step at which the oestrogen is extracted with sodium hydroxide from a solvent such as benzene or toluene.

*Reagents*

1. Phosphoric acid, s.g. 1.75, purest.
2. Crystalline oestrogens.
3. Ethanol, pure, redistilled from an all-glass still.

*Special Apparatus*

1. Test-tubes provided with ground-glass stoppers (capacity 15 c.c., Pyrex).
2. Fluorimeter provided with a micro-cuvette (capacity 2 c.c.).

*Procedure [195]*

1. A volume not exceeding 2 c.c. of the pure alcoholic solution of oestrogen is evaporated from a test-tube over a water-bath and oven-dried for 1 hour at 110° C.
2. To the dry residue, 3 c.c. phosphoric acid is added from a burette.
3. The test-tube, closed with ground-glass stopper, is transferred to a boiling water-bath (98° C.) and heated in the dark for 30 minutes, the mixture being shaken during the first 2 minutes of heating to ensure the solution of the oestrogen.
4. The reaction is interrupted by cooling in tap water.
5. The fluorescence is measured within an hour. The concentration of the oestrogen solution is read against a concurrently prepared standard reference curve.

Prolonged exposure of oestrogen in acid to light is avoided, for light interferes with the development of the fluorescence and also causes its gradual fading when developed. In performing the reaction, care is taken to prevent accidental entry of water (spray or vapour) into the reaction mixture, as water sharply depresses the reaction and exerts a marked quenching effect. The reaction vessels should be sealed with close-fitting ground-glass stoppers, rubber and cork being unsatisfactory.

Two modifications of this method have recently been described.

*Procedure [196]*

1. Carefully pipette the sample in 0.10 to 0.20 c.c. of ethanol or 0.10 c.c. of sodium hydroxide into the bottom of a fluorimetric test-tube.
2. Add 1 c.c. of 90 per cent phosphoric acid (10 c.c. water plus 90 c.c. phosphoric acid, C.P.). Shake the test-tube and place it for 10 minutes into a water-bath at 80° C. to develop maximum fluorescence.



3. After heating, add 6 c.c. of 65 per cent phosphoric acid (35 c.c. water plus 65 c.c. phosphoric acid, C.P.) to the tube and thoroughly mix with a corkscrew-shaped glass rod.
4. Read fluorescence on this diluted solution.

#### *Procedure [197]*

1. Place 0.4 c.c. of alcoholic solution of sample into test-tube.
2. Place tube in ice-bath and add 2 c.c. of concentrated phosphoric acid. Stir thoroughly (keeping tube in ice-bath).
3. Place tube in boiling water-bath for 6 minutes.
4. Remove from bath, add 8 c.c. of 25 per cent phosphoric acid (by volume) and reheat for 3 minutes.
5. Cool and read colour in a photometer using a 510 m.u. filter.
6. Calibration curves are prepared using crystalline oestrone.

Visually the red colour may not appear intense, since it is obscured by the green fluorescence. However, the intensity when measured with the photocolourimeter is as great as that obtained using the classical Kober phenol reagent. The main purpose of the phenol is to quench the green fluorescence.

### **Normal Values for Oestrogens**

#### *Menstruating Women*

In the normally menstruating woman excretion of oestrogens is found throughout the cycle except during the menstrual phase. A peak of excretion occurs at about the mid-cycle, and some observers report a second peak shortly before menstruation. According to Palmer [99], the peaks of excretion consist of oestrogen in the "free"-form. The following values for oestrogen excretion are reported:

#### *Blood*

- 25 m.u. per litre between the 7th and 14th day of the cycle.
- 50-100 m.u. per litre between the 21st and 28th day of the cycle [30].

#### *Urine*

- 50 m.u. per litre during the follicular phase.
- 300 m.u. per litre at the supposed time of ovulation [100].
- 1,000 I.U. per 24 hours at the peaks of excretion [91].

#### *Pregnancy*

The excretion of oestrogen in the blood and urine increases steadily during the first 8 weeks of gestation. After this point the

excretion rises rapidly to attain its maximum at term [92]. According to some investigators the rapid rise of excretion starts only after the seventeenth week of pregnancy [101]. During the first 8 months only a very small proportion of oestrogen is excreted in the "free" form; but a few weeks before the onset of labour the "free" oestrogen rises considerably, while the combined form diminishes, though it continues to make up the major part of the total urinary oestrogen [102].

According to Rakoff and others [103], the oestrogens of pregnancy serum are mainly bound to the protein fraction, because vigorous and prolonged hydrolysis is required to free the combined hormones, and protein-free filtrates are often free of any oestrogenic content, and nearly all the hormones can be recovered from the protein fraction. The oestrogens do not pass through a colloidal membrane and are not freed by triptic digestion.

The following are the excretion values observed:

*Blood:*

25-35 m.u. per litre until the 17th week, and thereafter 600-1300 m.u. per litre at term [101].

*Urine:*

300-600 m.u. per litre during the first 8 weeks [115], thereafter rising to 20,000 m.u. per litre at term.

200-1,000 I.U. during the first 8 weeks, 15,000-40,000 I.U. at term [104].

*Menopausal and Oophorectomized Women*

*Blood.*—No oestrogen has been demonstrated.

*Urine:*

6.8 m.u. per 24 hours during the menopause.

5.61 m.u. per 24 hours for women with flushes.

7.68 m.u. per 24 hours for women without flushes [105].

2-15 m.u. per litre in bilaterally oophorectomized women [106].

15-200 m.u. per litre in bilaterally oophorectomized women [107].

20 r.u. per litre in bilaterally oophorectomized women [108].

## Males

*Urine*

90-120 I.U. daily in the normal man [109].

2-17 m.u. per litre in the castrated male [106].

No cyclic variations have been demonstrated.

## PREGNANEDIOL

Pregnanediol is the excretion product of progesterone and is biologically inactive. Various methods of its determination are described. The original method suggested by Venning [41], for the isolation of sodium pregnanediol glucuronide does not show the free pregnanediol, for this is lost during extraction.

**Venning's Pregnanediol Assay**

1. An aliquot of a 24-hour urine specimen, which is expected to contain 20–40 mg. of the combined pregnanediol, is used.
2. The urine is extracted 4 times with a total of about one-third of its volume of normal butyl alcohol in a separating funnel (if a litre of urine is taken, the volumes of butyl alcohol are 200, 85, 50 and 50 c.c.).
3. The combined butyl alcohol extracts are centrifuged or allowed to stand until clear.
4. The supernatant butyl alcohol is transferred into a distilling flask, the precipitate is washed once with butyl alcohol and the washing added to the flask.
5. The butyl alcohol is evaporated to dryness under reduced pressure and the residue mixed with 60 c.c. of N/10 sodium hydroxide. This mixture is extracted 4 times with butyl alcohol (20, 20, 10, 10 c.c.). The butyl alcohol extract is twice washed with 5 c.c. of water, centrifugated, and the clear butyl alcohol evaporated to dryness in a 1-litre distilling flask under reduced pressure.
6. 5 c.c. of water are added to the flask with 10 c.c. of acetone. The mixture is warmed on a water-bath at about 50° C. until the precipitate is completely dissolved.
7. The mixture is transferred to a 125 c.c. Erlenmeyer flask. The original flask is washed out with acetone several times, and the volume of the mixture made up to 100 c.c. with acetone.
8. After allowing the mixture to stand overnight in a refrigerator at 5–10° C. a white precipitate settles at the bottom and most of the supernatant fluid can be drawn off by suction. The remainder is transferred to a 50-c.c. centrifuge tube, centrifugated and the acetone removed.
9. A few drops of water are added to the centrifuge tube and warmed on a water-bath. The original Erlenmeyer flask is washed out with hot 95 per cent ethyl alcohol and added to the centrifuge tube in order to dissolve the contents. The



hot alcohol solution is filtered with suction into a weighed beaker, evaporated to dryness on a water-bath and the contents weighed.

10. The first precipitate always contains over 10–30 per cent of impurities depending on the volume of urine and the amount of combined pregnanediol extracted. If the urine contains blood it is almost impossible to obtain a pure precipitate, but otherwise true quantitative values can be obtained by making a second precipitation with the acetone and water. In this event the first residue obtained on addition of acetone is simply re-dissolved in water and acetone is added. The amount of water used depends upon the amount of compound present, 2 c.c. being used for amounts less than 5 mg., 3 c.c. for amounts between 5 and 10 mg., and 5 c.c. for amounts over 10 mg., and an equal amount of acetone is added.
11. The calculated amount of sodium pregnanediol glucuronide is converted into terms of pregnanediol extracted per 24 hours as follows:

Example:

800 c.c. of a 24-hour specimen of urine of 1,500 c.c. volume are extracted. Two precipitations with water and acetone are carried out, 5 c.c. of water being used in each case. The weight of the residue is 25 mg. which represents 85 per cent recovery making the original amount 29.4 mg.

Sodium pregnanediol glucuronide =  $\frac{29.4 \times 1500}{800}$  mg.  
per 24 hours.

Pregnanediol extracts =  $\frac{29.4 \times 1500 \times 0.597}{800}$  mg. per  
24 hours.

### Venning's Method Modified

The following is a modification of this method which permits the recovery of both sodium pregnanediol glucuronide and free pregnanediol [42].

1. The amount of sodium pregnanediol glucuronide is determined by Venning's method. Until the first acetone precipitation the free pregnanediol is also extracted. After centrifugation sodium pregnanediol glucuronide precipitates

and the supernatant acetone contains the free pregnanediol. This is removed from acetone by the modified Weil's method.

2. The clear aqueous acetone is transferred from the centrifuge tube into a 125-c.c. Erlenmeyer flask and placed on a steam-bath until it has evaporated to dryness.
3. 10 c.c. of acetone and a few drops of N/10 sodium hydroxide are added and the flask gently warmed to dissolve the residue. While the mixture is kept warm more sodium hydroxide is introduced, a few drops at a time, until a total of 40 c.c. is added.
4. The mixture is slowly cooled and placed in an ice-box overnight. The free pregnanediol precipitates and is filtered out by suction.
5. The precipitate is washed with about 5 c.c. of water and a small quantity of hexane, and transferred back to the Erlenmeyer flask with hot acetone which is allowed to evaporate off on a steam-bath.
6. Further purification is carried out by dissolving in 5 or 10 c.c. of hot acetone and re-precipitating with 3 volumes of sodium hydroxide. It is again placed in an ice-box and filtered off.
7. The precipitate is washed with water and transferred to a 50-c.c. Erlenmeyer flask. A third precipitation from 2, 3 or 5 c.c. of ethyl alcohol by the addition of 2 volumes of water should complete purification. If necessary, precipitation can be repeated. Care must be taken to add water or sodium hydroxide very gradually, and the mixture is warmed gently on steam-bath when above precipitations are carried out.
8. The final precipitate is filtered off, transferred with ethyl alcohol to a small flask, and weighed after the alcohol has been evaporated off in a drying oven.

Other modifications [22, 43] of Venning's method, based on preliminary acid hydrolysis of the urine, permit recovery of pregnanediol entirely in the free form. (For details of test, see p. 262.)

Evaluation of the methods described is not possible since insufficient data are available. Venning's method is the only one used to a great extent, and the evidence supplied suggests that it is unreliable for the diagnosis of progestogen levels in gynaecological practice [44, 45]. In a study on 58 women, correlated with endometrial

biopsies, it was found that 42.8 per cent who had bleeding from progestational endometria excreted no pregnanediol, and that 62.5 per cent who had bleeding from oestrogenic endometria excreted pregnanediol in amounts equal to those excreting it in association with progestational bleeding [46].

### Normal Values

#### *Menstruating Women*

1-5 mg. per 24 hours' urine during the luteal phase, disappearing 1-3 days before menstruation [110].

5-8 mg. and occasionally 11 mg. daily [91].

#### *Pregnancy*

Between the 64th and 78th day (cessation of corpus luteum function) rarely above 20 mg. or below 6 mg. per 24 hours. Thereafter a gradual rise occurs in the pregnanediol excretion until about the 250th day, at which an average peak value of 85 mg. per 24 hours has been recorded [173].

4-10 mg. daily during the first 69 days of gestation, rising from the 80th to 100th day continuously, up to a maximum of 105 mg. daily by the eighth month [111].

It is suggested that the pregnanediol excreted during the first 3 months of pregnancy derives mainly from the corpus luteum and thereafter from the placenta.

## ANDROGENS

The assay of androgens is carried out on urine only, either biologically (by the comb-growth of the capon or baby chick, the latter being less reliable), or by the chemical reaction of 17-ketosteroids which are regarded as the excretion product of androgens. For the biological as well as the chemical assay, preliminary acid hydrolysis is required to free the androgens from conjugation.

### BIOLOGICAL ESTIMATIONS OF ANDROGENS

#### **Capon Comb Test [47]**

1. A 3-litre sample of urine kept fresh at 0° C. without preservative is made acid to pH 1 with concentrated hydrochloric acid (about 20 c.c. per litre when urine is fresh). Thereafter an additional 20 c.c. of hydrochloric acid per litre is added to the urine to render it strongly acid.
2. The mixture is poured into a flask fitted with a reflux condenser



leading to an arrangement for absorbing fumes in alkali, and made to boil within half an hour to 1 hour, and then boiled for 1 hour.

3. The urine is transferred to a continuous extraction apparatus and extracted with benzene for 20–24 hours. The benzene solution is freed from acid by extracting twice with 50 c.c. portions of saturated sodium bicarbonate solution, and the phenols removed by extracting twice with 50 c.c. portions of double-normal sodium hydroxide and then washing with water.
4. The benzene solution is evaporated to dryness and the residue extracted 4–5 times with 10 c.c. portions of redistilled ether.
5. The ether extract is filtered through a coarse sintered-glass filter and evaporated in small parts in a small, weighed flat-bottomed tube.
6. Arachis oil is added to the residue (100–300 mg.) sufficient to make the volume up to 3 c.c. The mixture is then heated and stirred until all the oil-soluble material has dissolved.
7. 0.1 c.c. of oil is injected daily for 5 days into each of 5 caponized leghorns. An average of 5 mm. increase in the length and height of the comb at the end of 24 hours after the last injection indicates a positive reaction.

For the less commonly employed method of assay which turns on comb growth in the baby chick, the reader is referred to the original paper by Frank [48].

### Normal Values

#### *Males*

7 mg. (70 I.U.) of androsterone daily [112].

15–170 I.U. per litre [113].

30–100 I.U. daily [114].

#### *Females (menstruating)*

The values in the female approach those in normal men [92, 114]. According to Hoffman [9], women with normal menstrual cycles excrete up to 50 I.U. of androgen daily.

### 17-KETOSTEROIDS

The 17-ketosteroids are excreted in the urine in combined form, and must first be freed by acid hydrolysis. Two types of 17-ketosteroid appear in the urine, the phenolic 17-ketosteroid, which is oestrone

and is removed by washing with alkali, and the non-phenolic 17-ketosteroid, which is the degradation product of androgens, deriving probably from both gonadal and adrenal cortical sources.

Numerous modifications of Zimmermann's colorimetric test [49], have been described [50, 51, 54-63, 116-20]. Preliminary hydrolysis to free the 17-ketosteroids from combination apparently transforms or destroys a significant proportion of the original steroid content of the urine [52, 53]. To avoid the deleterious effects of hydrolysis, simultaneous hydrolysis with extraction has been advocated [54, 51]. However, comparison with the method of hydrolysis before extraction has shown that independent hydrolysis yields significantly greater amounts of urinary 17-ketosteroids than obtained by simultaneous hydrolysis and extraction [55, 52]. Various solvents, usually benzene [56-9, 52], ether-toluol [52] and carbon tetrachloride [55, 60-2] are employed for extraction. Friedgood and others [52] demonstrated that the ether-toluol is superior to carbon tetrachloride extraction.

A number of colorimetric methods are described which differ in the strength of the alkali employed for developing the colour and in the amount of water in the reaction mixture, which in turn depends mainly on whether alcoholic or aqueous potash solution is used [60].

#### Wooster's Method [60]

1. The specimen is hydrolyzed for 15 minutes with a 10 per cent concentration of hydrochloric acid in a 2-litre Erlenmeyer flask under a large model of the single reflux condenser of Olson and Plass.
2. The specimens are cooled to 75° C. and extracted for 50 minutes in a Herschberg-Wolfe continuous extractor with 500 c.c. of carbon tetrachloride.
3. The carbon tetrachloride in the main body of the extractor is transferred to the boiling flask.
4. The boiling flask is fitted to a distilling head and the solution concentrated to approximately 150 c.c. on a sand-bath.
5. After cooling the specimen is washed 4 times with 10-c.c. portion of one double-normal sodium hydroxide solution delivered from an automatic pipette.
6. The rose-coloured pigment, with an absorption maximum of 520 $\mu$  is bleached out by shaking in a closed vessel with 10 c.c. of a 10 per cent solution of sodium hydrosulphite (Lycopon) in normal sodium hydroxide until the colour disappears.

7. The lycopon solution is removed and replaced by water for 10 minutes.
8. The water is removed and the carbon tetrachloride solution is concentrated to approximately 10 c.c. in a 300 c.c. boiling flask (also with a 35/20 spherical joint to fit the distilling heads).
9. The solution is then transferred to a 125 c.c. Erlenmeyer flask, and evaporated to dryness on a 70° C. water-bath, under reduced pressure.
10. The dry steroidal gums are diluted with 95 per cent alcohol to 10 c.c. in glass-stoppered volumetric flasks.
11. The colorimetric assay is made by the Holtorff-Koch method [58], using 5<sub>N</sub> aqueous potassium hydroxide and allowing 1 hour for colour development in a thermostatically-controlled water-bath maintained at 25 + 0.5° C.
12. 0.2 and 0.1 c.c. fractions of the sample are analysed. The colours developed have a high absorption at 420 $\mu$  which makes the use of a colour-correction equation impractical [63].

### Robbie and Gibson's Method

A rapid clinical determination of urinary 17-ketosteroids has recently been described by Robbie and Gibson [55]. The authors claim an accuracy with a maximum variation of about  $\pm 5$  per cent for any stage of the process and an over all variation of  $\pm 10$  per cent or less.

1. A 24-hour specimen of urine is collected and preserved by adding 10 c.c. of concentrated hydrochloric acid per litre.
2. 250 c.c. of the urine is placed in a 500 c.c. Erlenmeyer flask with a ground-glass connection to a reflux condenser. 25 c.c. of concentrated hydrochloric acid is added and the urine is hydrolized by boiling it on an electric hot-plate for 10 minutes. A glass bead is dropped in to prevent superheating. There is sometimes a tendency for the hot urine to overflow as it reaches the boiling point, but if the flask is set off the plate for a minute or so at the time boiling starts this can usually be avoided.
3. The flask is cooled slightly and 75 c.c. of carbon tetrachloride is added to it. The condenser is attached and the sample is refluxed for 10 minutes in a boiling water-bath on the hot-plate.
4. The carbon tetrachloride layer is drawn off by using a



separating funnel and is filtered by suction through one thickness of filter paper in a Buchner funnel.

5. The filtered carbon tetrachloride is washed once with about 20 c.c. of water, once with 20 c.c. of 10 per cent sodium hydroxide solution, and twice more with similar amounts of water. This may be done by transferring it back and forth from one separatory funnel to another. It is then placed in a 125 c.c. Erlenmeyer flask and evaporated to dryness on a steam-bath. A glass bead speeds up this evaporation.
6. 10 per cent of 95 per cent ethanol is added to the flask and the residue is dissolved by slight warming. If the 17-ketosteroid value is abnormally high a greater dilution may be necessary.
7. For the colorimetric determination 4 50 c.c. test-tubes are prepared as follows:
  - A. Standard solution: 1 c.c. of 0.2 mg./c.c. dehydroisoandrosterone solution in 95 per cent ethanol.
  - B. Unknown solution: 1 c.c. of the urine extract.
  - C. Unknown solution blank: 1 c.c. of urine extract plus 1 c.c. of 95 per cent ethanol.
  - D. Reagent blank: 1 c.c. of 95 per cent ethanol. 1 c.c. of 2.5N potassium hydroxide in 100 per cent ethanol is put in every tube, and to each tube, except the one containing the unknown solution blank (C), 1 c.c. of 2 per cent m-dinitrobenzene in 100 per cent ethanol is added. The alcoholic potassium hydroxide solution is made fresh each time it is used by grinding 7 grams of c.p. potassium hydroxide in 25 c.c. of 100 per cent ethanol, filtering the supernatant fluid to remove insoluble carbonate, adding another 25 c.c. portion of alcohol to the residue, which is ground again and then filtered. This grinding is done with a glass rod in a 50 c.c. graduate set in ice-water. The 2 per cent m-dinitrobenzene solution is made by placing 1 gram of the reagent in a 50 c.c. volumetric flask and filling to the mark with 100 per cent ethanol. The material dissolves more readily if the flask is slightly warmed.
8. The tubes are set in a dark place at about 25° C. and left for 45 minutes. They should be shaken occasionally.
9. 25 c.c. of 70 per cent ethanol is added to each tube and the contents mixed by inversion.

10. The light absorbed by each of the preparations is determined in a photoelectric colorimeter with a filter transmitting a peak intensity at about 5,200 Å. The instrument is set at zero absorption with a distilled water blank.\*
11. Calculation: The light absorption value for the urine extract (B) is corrected by subtracting the readings for the urine extract blank (C) and the reagent blank (D). The value for the standard solution (A) is corrected by subtracting the value for the reagent blank (D) only.

The concentration of the unknown may then be determined as follows:

Concentration of unknown,  $\text{mg}/22. = 0.2 / \text{corrected reading of unknown.}$

The 24-hour 17-ketosteroid excretion expressed as mg. of dehydroisoandrosterone — concentration of unknown, mg. per c.c.  $\times$  dilution of extract  $\times$  24-hour volume of urine in c.c./250.

Example:

24-hour volume of urine = 1,100 c.c.

Urine extract is diluted with 10 c.c. 95 per cent ethanol.

Reading of standard solution (A) = 55.6

Reading of unknown solution (B) = 47.6

Reading of unknown solution blank (C) = 2.3

Reading of reagent blank solution (D) = 8.6

Concentration of unknown

$$= 0.2 / 55.6 - 8.6 \times (47.6 - 8.6 - 2.3)$$

$$= 0.16 \text{ mg. per c.c.}$$

24-hour excretion

$$= 0.16 \text{ mg. per c.c.} \times 10 \times 1100 / 250 = 7.9 \text{ mg.}$$

17-ketosteroid as dehydroisoandrosterone per 24 hours.

### Warren's Method

An improved method has been described and modified by Warren [198].

1. Acidify with concentrated hydrochloric acid (15 c.c., S.G. 1.16), an aliquot (100 c.c.) of 24-hour urine, add 50 c.c. of benzene and reflux the mixture on a water-bath for 30 minutes.

\* A Fisher electrophotometer equipped with No. 525 filter and 23-c.c. cells is used in the determination. Ten readings of a 0.2 mg./c.c. dehydroisoandrosterone solution prepared as outlined showed a maximum variation of less than 0.5 per cent.

2. Cool the mixture, separate off the benzene layer and replace by 50 c.c. of fresh benzene. Then reflux again for 30 minutes.
3. Wash the combined benzene extracts successively with water (100 c.c.), 2N sodium hydroxide (4 times with 50 c.c. portions), N-hydrochloric acid (100 c.c.) and water (100 c.c.).
4. Filter the washed benzene solution through paper and distil the benzene off on a steam-bath. The last traces of solvent are removed by evaporation under reduced pressure.
5. Add to the dry residue absolute ethyl alcohol (4 c.c.) and dissolve by gentle warming.
6. Filter the alcoholic solution through paper and use 2/10 c.c. of alcoholic solution (equivalent to 5 c.c. of the original urine) for determination by colorimetry.

The colorimetric estimation is made by the method of Callow, Callow and Emmens [199].

### *Method of Colorimetry*

#### *Reagents*

- (a) Alcohol. Ordinary commercial absolute alcohol is used, the only further specification being that it should not have a content of aldehyde exceeding 0.0025 per cent.
- (b) m-Dinitrobenzene. A well-crystallized and fairly pure material (B.D.H. "extra pure", M.P. 89–89.5° C.) is taken and further purified as follows: 20 grams are dissolved in 750 c.c. of 95 per cent alcohol warmed to 40° C. and 100 c.c. of 2N sodium hydroxide are added. After 5 minutes the solution is cooled, and 2,500 c.c. of water are added. The precipitated m-dinitrobenzene is collected on a Buechner funnel, washed very thoroughly with water, sucked dry and recrystallized twice in succession from 120 c.c. and 80 c.c. of absolute alcohol. The material must be well crystallized in almost colourless needles, M.P. 90.5–91° C. Admixture of 1 per cent alcoholic solution with an equal volume of aqueous 2N sodium hydroxide should give no colour after an hour. The reagent is a 2 per cent w/v solution of this material in absolute alcohol. It is stored in a brown, stoppered bottle in the dark, and is stable for 10–14 days. In the actual colorimetric measurement the control solution without methyleneketone, should give a pale straw colour having a value of  $E_g = 0.20-0.21$  in a 1-cm. cell compared with alcohol.



- (c) Potassium hydroxide. The reagent solution is 2.5N potassium hydroxide in absolute alcohol. Nine grams of potassium hydroxide (B.D.H. "Analar" pellets) are dissolved with mechanical stirring in 50 c.c. of absolute alcohol, and the solution filtered through a hardened paper (Whatman No. 50) at the pump. The concentration is checked by titration of 0.5 c.c. with 0.1N sulphuric acid (methyl orange indicator) and the solution diluted with alcohol if necessary, to bring it within the limits of 2.48 and 2.52N. The solution is stable for 2-5 days if stored in a refrigerator. It must be discarded as soon as the faintest colour is perceptible.

### *Mode of Operation*

Test-tubes used for the reaction must have been cleaned with nitric and chromic acid mixture. Into one tube, to serve as "blank", are measured out in succession, from 1 c.c. pipettes graduated to 0.01 c.c., 0.2 c.c. alcohol, 0.2 c.c. m-dinitrobenzene solution and 0.2 c.c. potassium hydroxide solution. The solution of the test substance is measured out into a second tube, and there is then added sufficient alcohol to make the volume up to 0.2 c.c., followed by the reagents. The time of adding the potassium hydroxide is noted. The tubes are well shaken to disperse the dense potassium hydroxide solution, lightly stoppered, and placed in a water-bath kept at  $25 \pm 0.1^\circ \text{C}$  by means of a thermo-regulator. The tubes are shielded from all but dull, diffused light by a screen. After an hour, 10 c.c. of alcohol are added to each tube, and the contents mixed and transferred to the cells of the colorimeter, which are then closed by microscopic coverslips.

### **A Rapid Method for the Determination of Total Urinary 17-ketosteroids**

This has recently been reported by Drekter, Pearson, Bartzcak and McGavack [200].

#### *Method*

##### *Reagents*

Absolute ethyl ether (reagent grade). Absolute alcohol (commercial grade) is usually suitable but it is best to run a Zimmerman test blank before using.

m-Dinitrobenzene. Purify by heating in 10 per cent aqueous sodium hydroxide solution until melted. Decant while hot. Cool and wash twice with water. Add 95 per cent alcohol and dissolve by

warming. Keep temperatures below  $50^{\circ}\text{C}$ . Cool, add 5 volumes of distilled water and filter. Wash the precipitate twice with water and dry. Two per cent m-dinitrobenzene solution is made by dissolving 0.9 gram m-dinitrobenzene in 45 c.c. absolute alcohol. Run a Zimmerman test blank on the reagent before using. It may be necessary to crystallize from alcohol again if blanks are high.

Potassium hydroxide, 5N aqueous solution of electrolytic grade. Determine the normality with standard hydrochloric acid using methyl red as the indicator.

Sodium hydroxide. A solution of 10 grams reagent grade per 100 c.c. water.

Concentrated hydrochloric acid, reagent grade. 37.5 per cent hydrochloric acid by volume. The percentage of hydrochloric acid by volume is defined as the volumes of concentrated hydrochloric acid per 100 volumes of urine.

It is advisable to run preliminary Zimmerman tests on reagent blanks to determine the purity of reagents.

### *Procedure*

1. Place 10 c.c. of urine and 3 c.c. of concentrated hydrochloric acid in a 125-c.c. Erlenmeyer flask and stopper the flask with a Pyrex flathead stopper.
2. Heat the flask in a water-bath at  $80^{\circ}\text{C}$ . for 10 minutes, cool and transfer 5 c.c. of the hydrolysate to a 125-c.c. separatory funnel.
3. Add 20 c.c. ether and shake the funnel for 30 seconds. Remove the urine.
4. Wash the ether once with 10 c.c. of 10 per cent sodium hydroxide and once with 10 c.c. of distilled water; shake for 10 seconds with each wash.
5. Remove 5 c.c. of ether, evaporate the 5 c.c. and assay by means of the Zimmerman reaction. Similar aliquots can be taken for other assay methods such as the Pincus.

If a larger amount of "17-ketosteroids" is desired in the final extract, larger volumes of urine can be hydrolysed at  $80^{\circ}\text{C}$ . for 10 minutes using three parts of concentrated hydrochloric acid to 10 parts of urine. Volumes of hydrolysate greater than 10 c.c. can be extracted and washed with proportionately larger amounts of ether, 10 per cent sodium hydroxide and water. Similarly, volumes of urine less than 10 c.c. can be assayed using proportionately smaller volumes of acid and ether to hydrolyse and extract the "17-ketosteroids".

The colorimetric assay is made by the Zimmerman method. Add 0.2 c.c. absolute alcohol, 0.2 c.c. m-dinitrobenzene solution and 0.13 c.c. of 5N sodium hydroxide solution to the dried extract. Keep the solution in the dark in a water-bath whose temperature is 27° C. for 90 minutes. After 90 minutes dilute the solution with 1 c.c. of diluent; the diluent consists of 3 parts of absolute alcohol to 1 part of water. Read the diluted solution in a colorimeter using a green filter.

Prepare the standard as directed above, substituting 0.20 c.c. of a solution of dehydroisoandrosterone in absolute alcohol (1.0 microgram hormone per 0.01 c.c. alcohol) for the 0.20 c.c. absolute alcohol and omitting the ether extract.

Prepare the urine blank as above, adding 0.20 c.c. absolute alcohol in place of 0.20 m-dinitrobenzene solution.

Prepare method blanks by substituting water for urine in the hydrolysis and extraction; then proceed as above.

Prepare the Zimmerman reagent blanks as above, omitting the ether extract.

## ADRENAL CORTICOIDS [201]

### BIOLOGICAL ASSAY FOR GLYCOGENIC CORTICOIDS

Male white mice from the same colony, weighing 20–25 grams, were used. As the response to adrenal cortical extracts may vary with different strains of mice, it is important to keep to the same strain. Two days before adrenalectomy the mice are removed from their stock fare of Purina dog chow and placed on the McCollum diet which contains 26 per cent protein and 52 per cent carbohydrate. The animals are anaesthetized with nembutal or ether and are adrenalectomized by the usual lumbar route. Following adrenalectomy they are placed in a constant-temperature room or box at 76° C. and are maintained on the McCollum diet. 0.9 per cent sodium chloride containing 5 per cent glucose is given as drinking water on the first post-operative day. This solution is removed on the morning of the second post-operative day and 0.9 per cent sodium chloride is substituted throughout the remainder of the test. The glucose administration immediately following adrenalectomy almost completely eliminates the mortality due to operative shock. On the third post-operative day food is removed at 5 p.m. and the mice are fasted until the following morning at which time the drinking water is also removed. On the fourth post-operative day, beginning at 9.15 a.m., a total of seven injections is given at 9.15 a.m., 10 a.m., 10.45 a.m., 11.30 a.m., 12.30 p.m., 1.30 p.m. and 2.30 p.m. The material to be tested is taken into solution in 5 per cent



glucose and 10 per cent alcohol. 0.20 c.c. is given subcutaneously for each injection so that each mouse receives a total of 1.4 c.c. extract containing 70 mg. glucose. At 3.30 p.m. the mice are weighed and anaesthetized with sodium amytal (0.2 c.c. of a 1.8 per cent solution). The livers are quickly removed and plunged into 4 c.c. of hot 30 per cent potassium hydroxide contained in a 15-c.c. graduated centrifuge tube. The tubes are heated in a boiling water-bath and frequently shaken until all the tissue is in solution. The glycogen is precipitated by the addition of 1.2 volumes of 95 per cent alcohol. The tubes are heated until the mixture just begins to boil, cooled in an ice-bath and centrifuged. The supernatant liquid is poured off and the tubes are allowed to drain. The sides of the tubes are washed down with 0.5 c.c. alcohol and again allowed to drain. Final traces of alcohol are expelled by heating the tubes for a few minutes in the hot water-bath.

The glycogen is hydrolysed and the glucose is determined by the method of Good, Kramer and Somogyi. The glycogen is expressed in terms of milligrams of liver glucose per 100 grams of mouse body weight.

### *Standard*

The reference standard is 11-dehydro-17-hydroxycorticosterone (Crystalline Compound E of Kendall). The biological activity equivalent, that of 1 microgram of 11-dehydro-17-hydroxycorticosterone in terms of amount of glycogen deposited, is defined as one glycogenic unit.

### *Preparation of Urinary Extracts*

For urines containing a normal or low titre of glycogenic activity a complete 48-hour specimen is necessary for the assay; for urines containing a high titre a 24-hour specimen is sufficient. The urine is adjusted to pH 1 with hydrochloric acid or sulphuric acid and extracted three to four times with ethylene dichloride: chloroform may also be used. If any emulsions are formed they can be broken by centrifugation or by allowing the mixture to stand for an hour. The clear ethylene dichloride extract is evaporated almost to dryness under reduced pressure; the temperature of the water-bath should not exceed 55° C. The residue is taken up in 30 c.c. chloroform and the chloroform is extracted 3 times with 5 c.c. of cold N/10 sodium hydroxide and 3 times with water. These washings are re-extracted with chloroform. The combined chloroform is evaporated down to a volume of approximately 1-2 c.c. and is transferred into a test-tube with small amounts of chloroform. The test-tube is placed in a water-

bath at  $50^{\circ}$  C. and the remainder of the chloroform is removed under a stream of nitrogen. The dry residue is stored in the cold until ready for assay.

### *Preparation of Extract for Assay*

Six to eight mice must be used for each assay. For normal male urine the equivalent of 6 hours of urine is administered to each mouse, whereas for female urine or urines expected to be low in glycogenic activity, the equivalent of 8 hours of urine is given to each animal.

The following will illustrate the manner in which the residue is prepared for assay so that the final extract will contain 10 per cent alcohol plus the required amount of glucose which is 70 mg. per mouse. For example, the residue obtained from a 48-hour specimen of urine is to be assayed on eight mice. Each mouse receives 7 injections of 0.2 c.c. so that the final extract is made up to  $7 \times 0.2 \times 8 = 11.2$  c.c. and should contain  $70 \times 8 = 560$  mg. of glucose. A 10 per cent glucose solution is accurately prepared and 5.6 c.c. of this solution are measured into a 15-c.c. graduated tube. The urine residue is dissolved in 1.12 c.c. of alcohol and this solution is run slowly drop by drop from a pipette into the 10 per cent glucose solution, the tube being shaken continuously during the addition of this material. The original test-tube and pipette are rinsed out with water which is added to the extract and the final volume is made up to 11.2 c.c. with water. The extracts are stable for 24 to 48 hours at ice-box temperatures.

Modifications of this method have been reported recently [202, 203].

## THE "COLD TEST"

### A METHOD FOR THE ASSAY OF ADRENAL CORTICAL STEROIDS [204]

White mice of unknown strain which were employed were of male sex except in one experiment as noted below. Albino male and female rats 22-24 days of age and weighing between 35 and 52 grams were used. The bulk of the rats were obtained from Sprague-Dawley, Madison, Wisconsin, and were bilaterally adrenalectomized the day after arrival. The experiments were run between 12 and 24 hours after operation.

The animals, both mice and rats, were exposed to  $5.5 \pm 1.5^{\circ}$  C. for the duration of the experiment. Groups of 100 animals were usually run simultaneously, at least ten of which served as controls.

During the course of the experiment the animals were given no food or water and were kept in the dark except for a brief period of light every 30 minutes at which time observations were made. Each animal was housed in an individual wide-mouth glass jar containing a sheet of filter paper for bedding. In experiments requiring two injections during the course of the experiment, these injections were done in the cold room.

Oral administration was performed by means of a stomach tube immediately before the animals were placed in the cold room. Rats were not anaesthetized, but mice were subjected to light ether anaesthesia during this treatment. Rats received the total dose to be tested in 1 c.c. of 10 per cent ethanol while mice received 0.5 c.c. of a similar solution. In some experiments on mice the material was dissolved in oil and injected subcutaneously.

Death was considered to have occurred when the animal had no visual signs of respiration, showed low body temperature, and on stimulation showed no responsive movements. This stage was easily discernible in mice but more difficult in rats.

Extracts of men's urine were prepared with ethylene dichloride as a solvent and partial purification was accomplished by removal of inactive materials with alkali [202].

Between the fourth and twelfth day after operation all animals died of adrenal insufficiency. The mean survival was 7.5 days. A pooled sample of normal male urine contained the equivalent of 0.6 mg. of 11-dehydrocorticosterone per litre.

This test is simple, rapid and potentially very sensitive, but its usefulness is limited by an erratic variation in sensitivity of animals from group to group.

### Normal Values for 17-ketosteroid Excretion

#### *Females:*

5.1 to 14.2 mg. per 24 hours.

Average 9.0 mg. per 24 hours [64].

#### *Males:*

8.1 — 22.6 mg. per 24 hours

Average 13.8 mg. per 24 hours [64].

Daily variations 5 mg. [64].

Daily variations  $\pm$  40 per cent [65].



*Children under 10 years: values approximating 0*

12 years: at least 1 mg. daily

18 years: approximately 9 mg. daily [66].

Only considerable deviations from the normal are of clinical significance.

## URINARY CHLORIDES

### Cutler-Power-Wilder Test [67]

The patient is given a low salt diet, containing 0.59 gram of sodium, 0.95 gram of chloride and 4.1 gram of potassium.

On the afternoon of the first day and the morning of the second day the patient receives orally 42 mg. potassium citrate per pound of body weight. Water intake is generous. On the second day the liquid intake is set at 18 c.c. per pound body weight, and on the third day, between 8 a.m. and 11 a.m., at 9 c.c. per pound of body weight. Urine is collected from 8 p.m. of the second day to 8 a.m. of the third day, and from 8 a.m. to 12 noon of the third day. Urine chloride determination is made according to the standard method.

At the end of the examination an intravenous infusion of the following is given:

Glucose.	.	.	.	.	.	.	50 grams
Sodium chloride	.	.	.	.	.	.	10 grams
Sodium citrate	.	.	.	.	.	.	5 grams
Eucortone	.	.	.	.	.	.	20 c.c.

in 1,000 c.c. of sterile water.

Extreme caution is necessary in carrying out this test since severe cortical failure may precipitate a dangerous adrenal crisis.

### *Normal Values*

Concentration of urinary chloride is less than 125 mg. per 100 c.c. of urine.

## THE "WATER TEST" [205, 206]

### A DIAGNOSTIC PROCEDURE IN ADDISON'S DISEASE.

#### Procedure I (based on volume of urine)

On the day before the test the patient eats three ordinary meals but omits extra salt. He is requested not to eat or drink anything after 6 p.m. Until this time he may drink water as desired. At 10.30 p.m.

he is requested to empty his bladder and discard the urine. All urine passed from then on until and including 7.30 a.m. is collected. The volume of this urine is measured and it is saved for chemical analysis. Breakfast is omitted. The patient is asked to pass urine again at 8.30 a.m. and immediately thereafter is given 20 c.c. of water per kilogram of body weight (9 c.c. per pound). He is asked to drink this within the next forty-five minutes. At 9.30, 10.30 and 11.30 a.m. and 12.30 p.m. he is requested to empty his bladder. In order to eliminate the effects of exercise and posture on urinary excretion, he is kept at rest in bed except when up to pass urine. Each specimen is kept in a separate container. The volume of the largest one of these four specimens is measured. Under these conditions some patients having Addison's disease excrete so little urine that they cannot pass it more than once or twice during the entire morning.

*Inferences that may be drawn from Procedure I*

(1) If the volume of any single hourly specimen passed during the morning is greater than the volume of urine passed during the night, the response to the result is negative; that is, such a response indicates the probable absence of Addison's disease. (2) If the volume of the largest hourly specimen passed during the morning is less than the volume of urine passed during the night, the response to the test is positive; that is, Addison's disease may or may not be present.

### Procedure II

**(based on chemical composition of blood and urine)**

To carry out this procedure blood is drawn (preferably under oil) while the patient is still fasting, and the plasma is analysed for its content of urea and chloride. The specimen of urine which was passed during the night is also analysed for urea and chloride. From these four determinations, and from the results obtained from Procedure I, the following equation is solved:

$$A = \frac{\text{Urea in night urine (mg. per 100 c.c.)}}{\text{Urea in plasma (mg. per 100 c.c.)}} \times \frac{\text{Chloride in plasma (mg. per 100 c.c.)}}{\text{Chloride in night urine (mg. per 100 c.c.)}} \times \frac{\text{Volume of day urine (c.c.)}}{\text{Volume of night urine (c.c.)}}$$

The term "day urine" applies to the largest of the hourly specimens passed during the day; "night urine", to the entire amount which was passed from 10.30 p.m. to 7.30 a.m. It is immaterial how

these values are expressed, provided that the same method is used throughout the equation. For example, if the concentration of plasma chloride is expressed as milligrams of sodium chloride per 100 c.c. the concentration of urinary chloride should be expressed in the same manner.

### *Inferences that may be drawn from Procedure II*

(1) If the value of A is greater than 30, the patient is probably not suffering from Addison's disease. (2) If the value of A is less than 25, the patient probably has Addison's disease, provided that nephritis has been excluded.

If the results of Procedure II are not all equivocal or if they are not indicative of Addison's disease when there is strong clinical evidence to the contrary, the test devised by Cutler, Power and Wilder [67] may be conducted. This can be instituted immediately without loss of any of the patient's time, since the day of the "water test" can constitute the first day of the provocative test.

### PERI-RENAL INSUFFLATION [68]

The patient is placed on his side in the ordinary position for exposure of the kidney, with sandbags under the loin so as to increase the space between the twelfth rib and the iliac crest. The patient is then rotated forward to allow the peritoneal contents to fall away as much as possible from the site of injection. Both the twelfth rib and the outer edge of the erector spinae muscles are outlined with mercurochrome; an acute angle is thus formed by the junction of these two lines. After preparing the skin for the surgical procedure, a small amount of cocaine hydrochloride is injected. An ordinary spinal tape needle is introduced at the angle of the red lines, in a direction pointing slightly upward towards the twelfth rib and somewhat forward and away from the erector spinae muscles. The needle is introduced until Gerota's fascia has been pierced; this usually proceeds without difficulty. Aspiration is attempted, to be sure that the needle has not entered a blood-vessel. The needle is then attached to a two-bottle pneumothorax outfit, with two glass attachments for washing and filtering air. Air is delivered, doubly filtered through cotton, and washed by a 1 : 500 mercury bichloride solution under 6 inches of gravity pressure. If the proper place has been entered the air will bubble freely under this pressure. From 300 to 500 c.c. of air should be introduced, the larger volume producing better visualization.



After removing the needle the patient is made to sit up and perform "rowing" exercises for about 10 minutes. Manual massage over the kidney region can be employed with similar results.



FIG. 16.—PERI-RENAL INSUFFLATION

The X-ray pictures are taken preferably in the oblique or in the antero-posterior position. Lateral views are of lesser value. Combination of the air insufflation technique with intravenous or retrograde pyelography gives the best results (see Fig. 16).

### ENDOMETRIAL BIOPSY

Oestrogen and progestogen activity are reflected in the endometrium, and biopsies carried out repeatedly or at a certain stage of the cycle indicate ovarian function. Biopsies are usually made the first 12–18 hours after the onset of bleeding, so as to show the true state of endometrial differentiation and to avoid disturbing a possible early pregnancy. In cases of infrequent menstruation with intervals of long duration, or in prolonged uterine bleeding, endometrial biopsy may be performed at any time.

Neither anaesthetics nor analgesics are usually needed; but the patient benefits by morphia  $\frac{1}{4}$  grain, or by pethidine hydrochloride 25–100 mg. orally. The instrument used is a suction curette or small punch curette, by which portions of endometrium are removed from the anterior, posterior and lateral walls as well as from the upper portion of the uterus. Slight preliminary dilatation of the cervix may be necessary. The tissues are embedded in paraffin and the sections stained with haemotoxylin.

### TEST FOR OVARIAN RESPONSIVENESS

Hamblen advocates the following schedule for a test of gonadotrophic therapy in oestrogenic ovarian failure:

Equine gonadotrophin 400 I.U. intramuscularly daily for 10 days.

Chorionic gonadotrophin 500 I.U. intramuscularly daily for 10 days.

No treatment from the 4th to the 8th week.

In the absence of ovarian response at the end of this period, the schedule is repeated with doses increased to 1,000 I.U. for each of both hormones.

If the ovaries fail to respond at the eighth week, another diagnostic treatment schedule is carried out with doses increased to 1,500 I.U. for each of the gonadotrophins.

If no evidence of ovarian stimulation is obtained at the eighth week of the cycle, the ovaries are considered to be non-responsive.

### VAGINAL SMEAR

The vagina undergoes cyclic changes in response to the ovarian hormones.

### Oestrogens

Oestrogens induce hyperplasia of the vaginal epithelium and bring about a deposition of glycogen in its intermediate and superficial layers. The amount of glycogen in the vaginal cells at different stages of the menstrual cycle is thus directly proportional to the oestrogen activity of the ovary [147]. Proliferation of the vaginal mucosa under the action of oestrogens produces a progressively

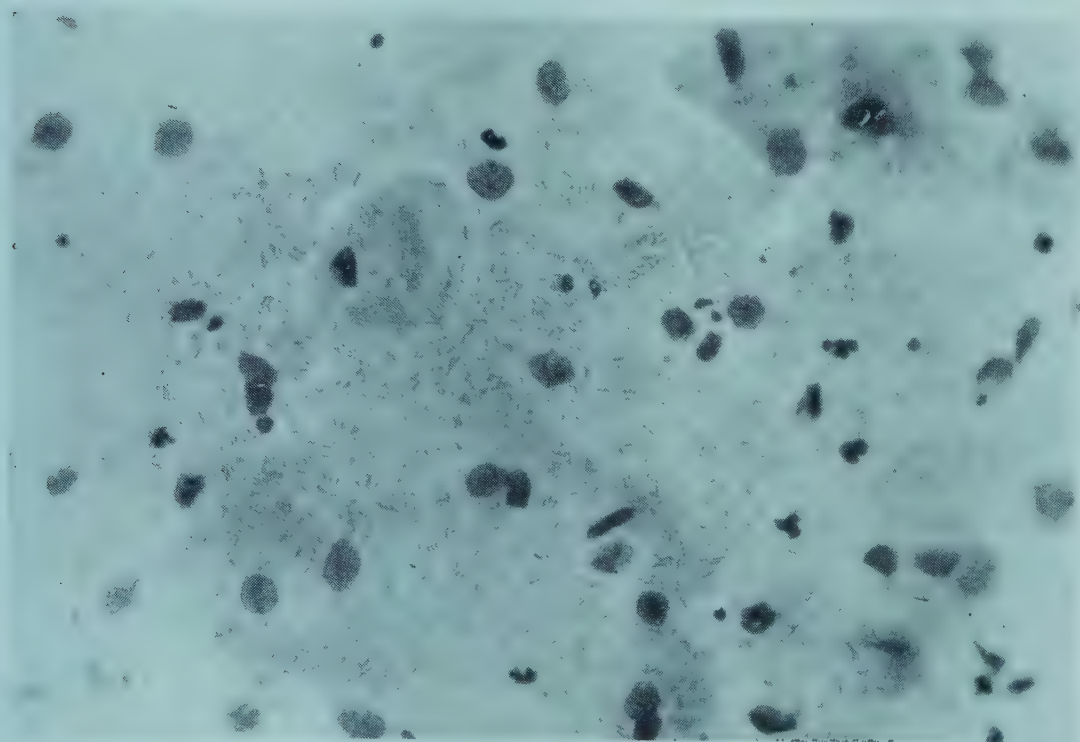


FIG. 17.—VAGINAL SMEAR. COMPLETE CYTOLYSIS

increasing density of the nuclei in the epithelial cells, and replacement of the normal cytoplasm by a keratohyalin protein similar to the horny substance of finger-nails or hair. Thus, the degree of epithelial cornification corresponds with the oestrogenic content (see Fig. 20).

At a certain level oestrogens may produce varying degrees of cytolysis, causing complete destruction of the cytoplasm without affecting the nuclei [219]. (See Fig. 17.)

### Progestogen

In the absence of oestrogen progestogen appears to have little effect upon the proliferation of the vaginal epithelium [148]. When oestrogen is present, however, and produces epithelial proliferation, progestogen



has an inhibitive effect [149], leading to the desquamation of the mucosa and aggregation of the proliferated cells. The more progesterone there is present, the greater is the desquamation and aggregation of cells seen in the vaginal smear [74]. (See Fig. 21.)

Progestogen does not influence the glycogen content in the cells.

### Androgens

Androgens produce increased mucification of the vaginal mucosa [207-9] but unless abnormally increased do not inhibit cornification in presence of oestrogens. In fact, the antagonism at slightly increased levels seems to be directed rather against the corpus luteum hormone than to oestrogens, since it inhibits desquamation and thus permits the cells to cornify under the action of oestrogens. This is

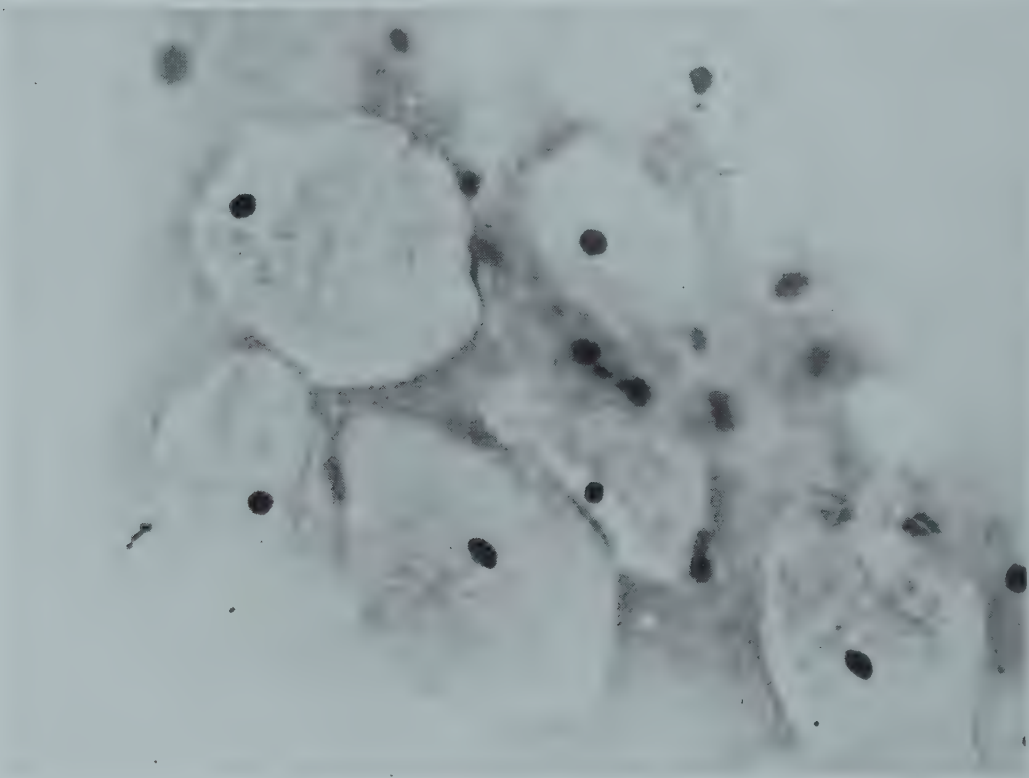


FIG. 18.—VAGINAL SMEAR. MUROID-CORNIFIED TYPE

most likely the explanation of the mucoid cornified type of smear which may occur during pregnancy and other conditions when a shift in the androgen-oestrogen ratio occurs towards an absolute or relative increase in androgens [210]. (See Fig. 18.) In large and protracted doses androgens inhibit both epithelial proliferation and glycogen deposition [178].

## CHARACTERISTICS OF VAGINAL SMEAR

**Cyclic changes**

The following changes occur at the various stages of the menstrual cycle:

*(a) Post-menstrual Phase*

Epithelial cells exhibit predominantly large, round, vesicular nuclei and granular cytoplasm. In addition, many leucocytes and bacteria as well as some thick mucus may be present (see Fig. 19).

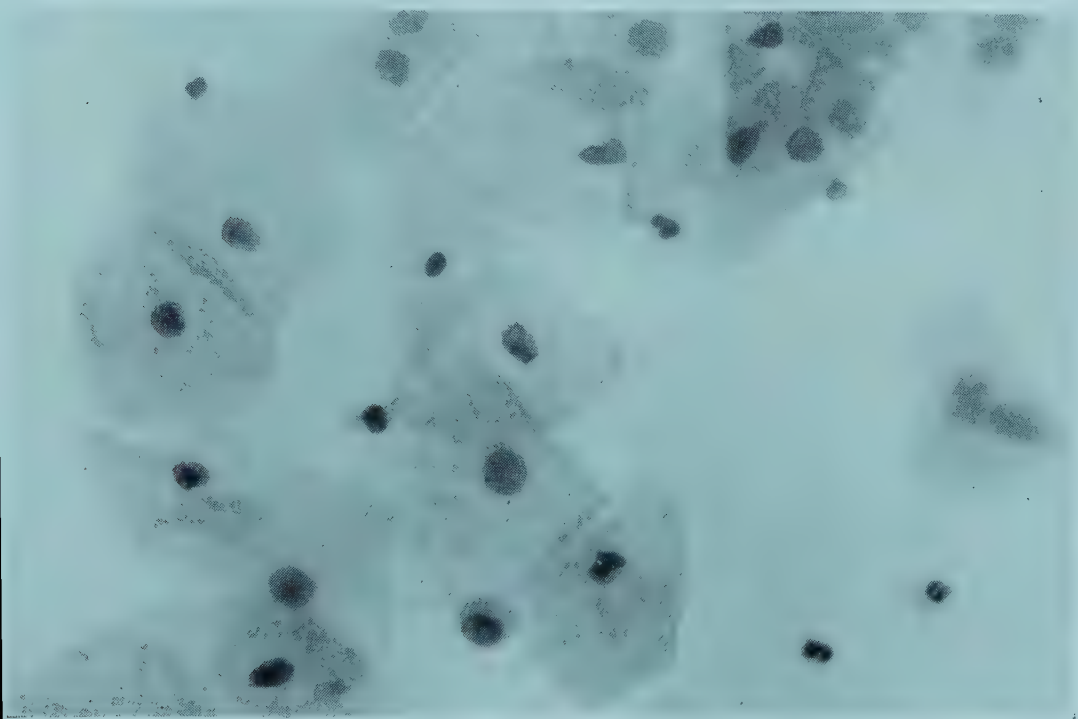


FIG. 19.—VAGINAL SMEAR. FOLLICULAR PHASE

*(b) Pre-ovulatory Phase*

Increased proliferation of vaginal mucosa. The superficial cells show more cornification. The nuclei of the epithelial cells become smaller, stain deeper and are pyknotic; the cytoplasm has lost its granular contents, is clear and the edges are distinctly defined. Bacteria and leucocytes gradually disappear from the picture (see Fig. 20).

*(c) Ovulatory Phase*

Sudden decrease in oestrogen secretion, which is often reflected by the appearance of some red blood cells in the smear. In addition,

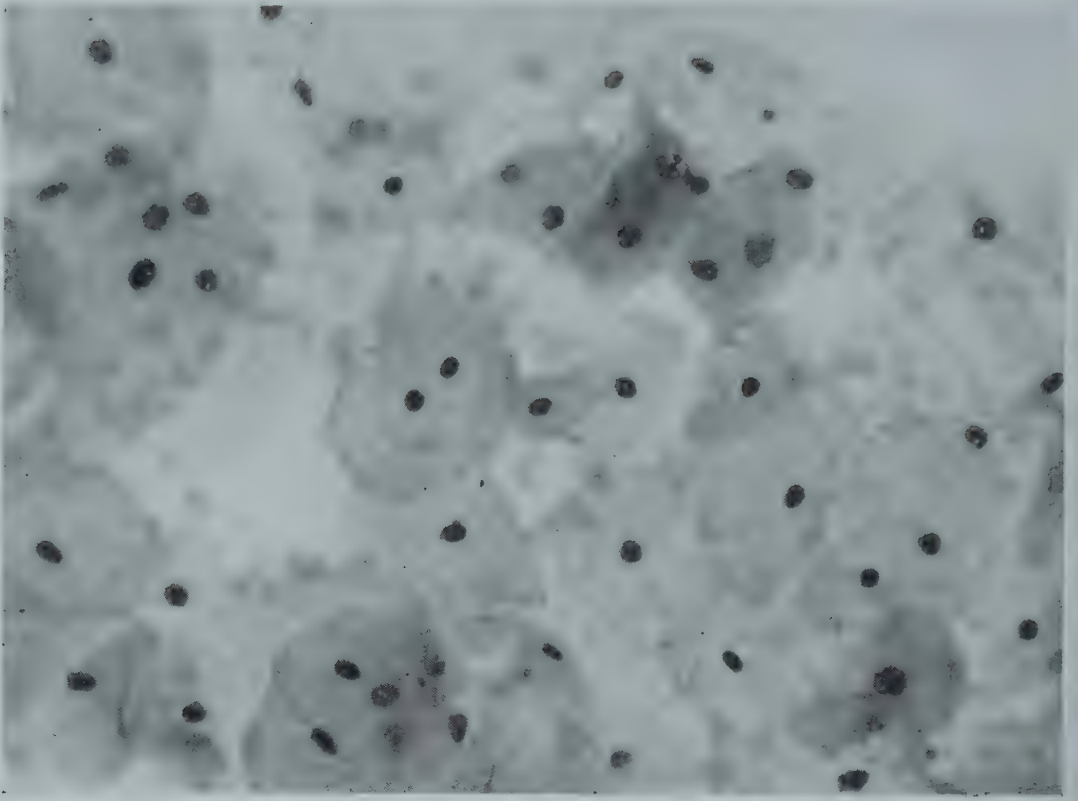


FIG. 20.—VAGINAL SMEAR. LATE FOLLICULAR PHASE

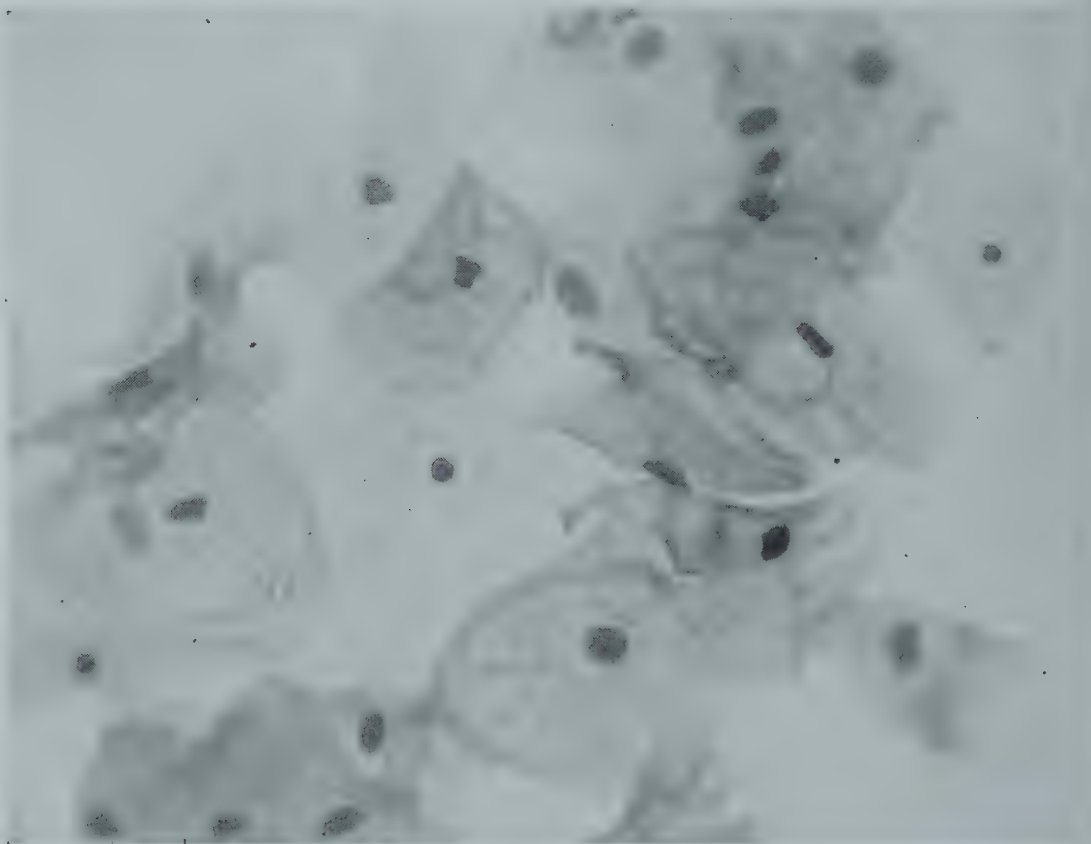


FIG. 21.—VAGINAL SMEAR. POST-OVULATORY PHASE



there is marked desquamation of cornified cells; there are very few leucocytes and bacteria and there are some cornified and precornified cells with folded edges.

*(d) Post-ovulatory Phase*

Continued desquamation of cells which are characteristically crumpled and folded together. Several types of epithelial cells are present. Some are completely cornified; the cytoplasm is wrinkled, scalloped edged and agranular, while the nuclei are small and dense. These cells decrease in number as the luteal phase advances. Other cells show large and oval nuclei with a wrinkled cytoplasm and scalloped edges. Their cytoplasm is granular and occasionally vacuolated. This type is supposedly suggestive of progestin activity. There is marked influx of leucocytes and bacteria. The mucus remains abundant (see Fig. 21).

*(e) Pre-menstrual Phase*

Cells with larger, more vesicular nuclei. There is an abundance of leucocytes, bacteria and mucus. The cells slough fragments off.



FIG. 22.—VAGINAL SMEAR. LATE LUTEAL PHASE

Their nuclei may be vesicular or pyknotic. The cells may be broken into halves, with one part containing the nucleus. The cytoplasm may

show partial cornification and the presence of granular vacuoles. In addition, a few red blood cells, many leucocytes, much mucus, many bacteria and much debris are present. The smear, on the whole, is termed "dirty" (see Fig. 22).

*(f) Menstrual Phase*

Blood and mucus. Epithelial cells show earliest phase of follicular development.

**Vaginal Glycogen Variations**

Rakoff [181], in a study of 372 smears from 37 women, found no glycogen during the first week of the cycle, but a rising glycogen concentration at about the mid-cycle. During the luteal phase a further increase was noted reaching a peak in the last week of the cycle. A very distinct fall in glycogen concentration commonly occurred a few days before the onset of menstruation.

**Vaginal Smears in Pregnancy**

A highly-differentiated cell appearing in the smear of pregnant women was first described by Papanicolaou [75], and termed "pregnancy cell". The formation of intracellular vacuoles in the pregnancy smear may, however, occur in certain cases of amenorrhoea or may be inhibited in the presence of pathological changes in the vaginal mucosa [76]. Likewise, complete glyopenia may occur in cases of vaginitis and febrile states [77].

In a study on 97 women throughout pregnancy Hall [78], found that during the first 2-4 weeks the degree of vaginal cornification remained rather high, but thereafter dropped in most cases to a 4 + phase (highest oestrogen phase 7 + ). Threatened abortions were connected with a vaginal smear of high oestrogenic value; but in no case in which the vaginal smear stayed at 3 + or lower was there any disturbance. The author concludes that a high cornified vaginal smear is a usual indication of approaching difficulty. Schuman [79], on the basis of smears from approximately 350 patients in all stages of pregnancy, suggests that pregnancy may be diagnosed if there is pronounced cornification. The highest peak of cornification was seen in a case of hyperemesis, and the amount was definitely increased in a patient with early hypertensive toxæmia.

Recent studies [210, 219, 220] of cytological changes during pregnancy have shown that the vaginal smear during the first two to three weeks of gestation is marked by an accentuation of the mid-luteal phase (see Fig. 23). Pregnancy may often be suspected on the basis

of such smears. As pregnancy advances the number of cells decreases with increase in cellular size. At about the twelfth week of pregnancy only a few "luteal cells" (cells with rod-shaped pyknotic nuclei, normally occurring in small numbers during the luteal phase) are present. The nuclei are large, round or oval, and vesicular, and leucocytes are usually found (see Fig. 24). With advancing pregnancy the number of cells increases with a decrease in cellular and nuclear

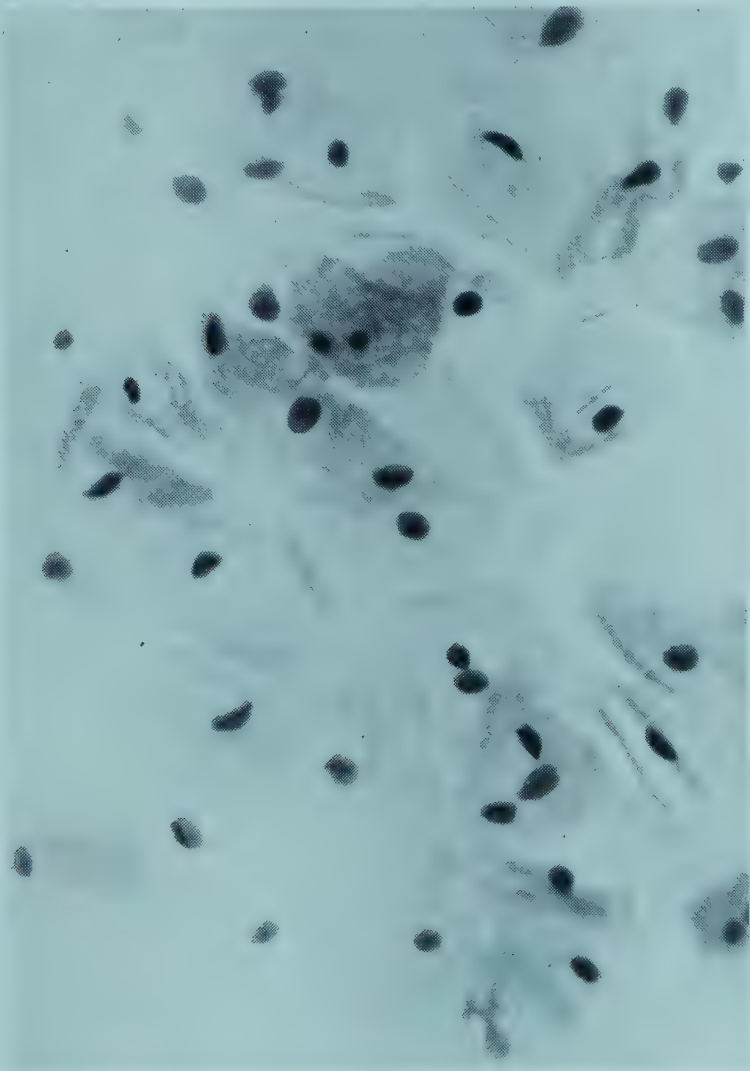


FIG. 23.—VAGINAL SMEAR. EARLY PREGNANCY

size. There is no increase in the number of luteal cells and the leucocytes disappear (see Fig. 25). At the later stages the total number of cells increases further with decrease in cellular size and increase in the number of luteal cells (see Fig. 26). In addition, three specific types of smears may be encountered. These are the *cytolytic* (see Fig. 17), the *mucoïd-cornified* (see Fig. 18) and the *glycolytic* smears.



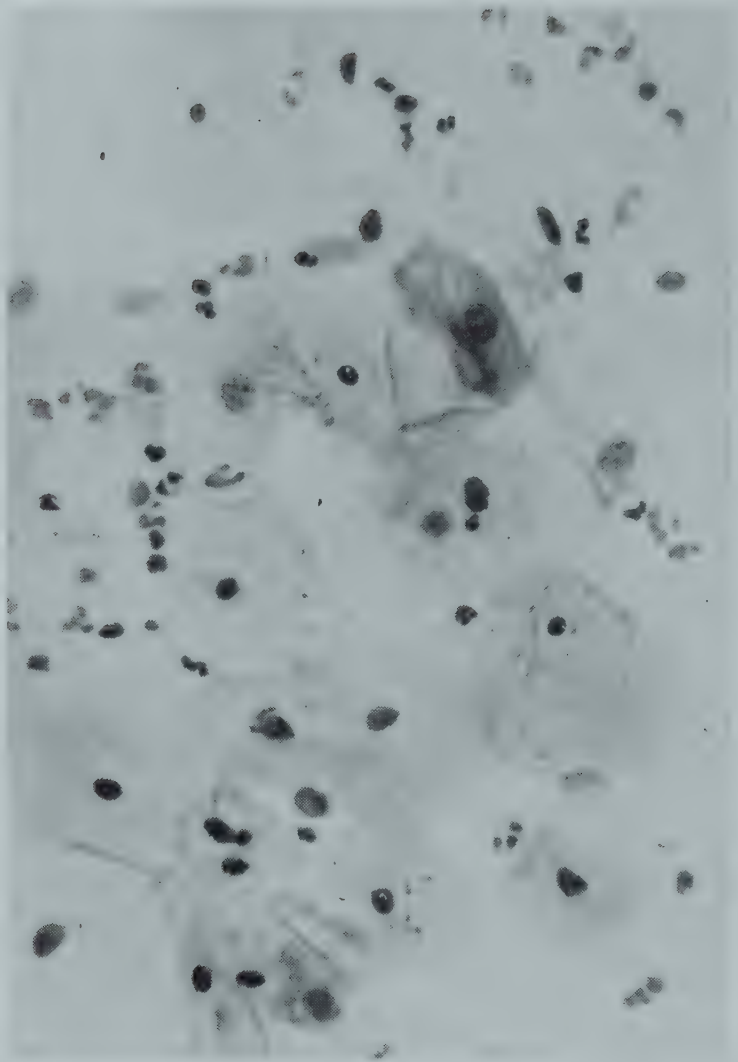


FIG. 24.—VAGINAL SMEAR. PREGNANCY 12 WEEKS

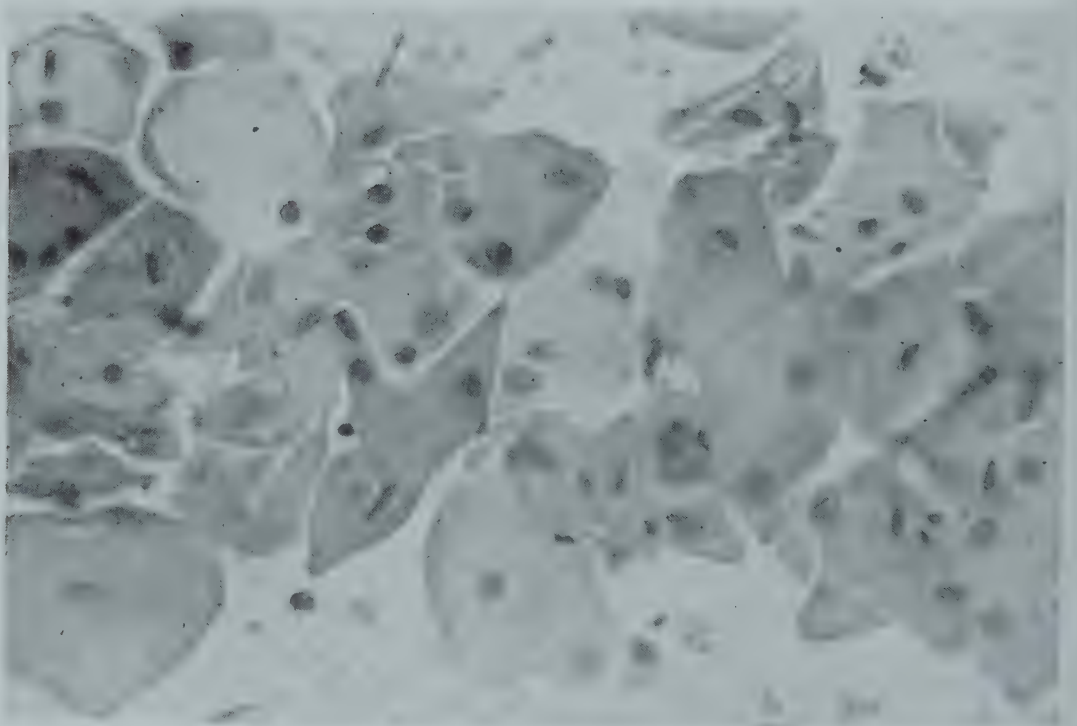


FIG. 25.—VAGINAL SMEAR. PREGNANCY 20 WEEKS

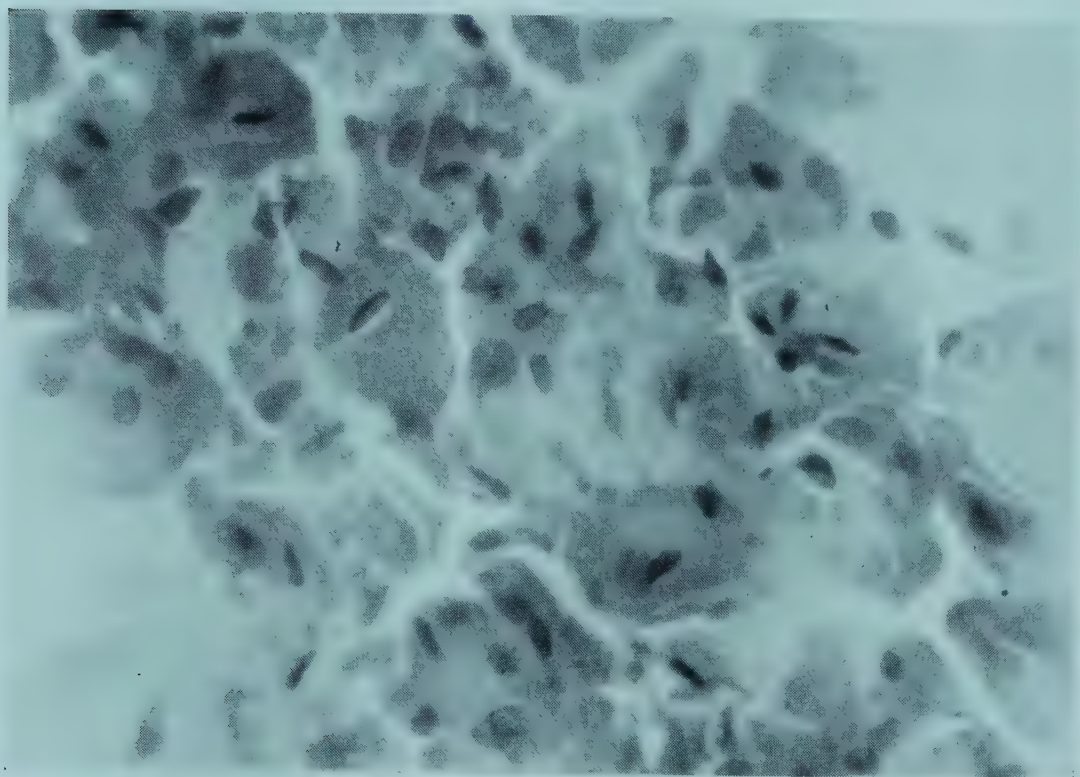


FIG. 26.—VAGINAL SMEAR. PREGNANCY 36 WEEKS

### **Menopause**

At the menopause atrophy of the genital mucosa begins. The cornified cells tend to disappear and there is less glycogen deposition. During the menopause the cells become larger in size with a progressive increase in nuclear size. After the menopause, cellular size decreases with an increase in nuclear size, the nuclei finally taking up about 50 per cent of the whole cell (see Figs. 27 and 28).

### **Vaginal Smear for Carcinoma**

Smears are stained by Papanicolaou's method [80], or its modification [81], which show marked cellular differentiation.

The characteristic features common to vaginal smears from cervical carcinoma are as follows [69-71, 80, 177, 212-14].

#### *Differentiated Type*

The characteristic features of the smear from the cervical canal in this case are: bizarre cellular forms of varied size and shape representing amoeboid, tadpole, saddle-back and other irregularly-shaped cells; elongated cells resembling muscle-fibre cells may be found. These cells contain an elongated hyperchromatic nucleus or two or three nuclei.

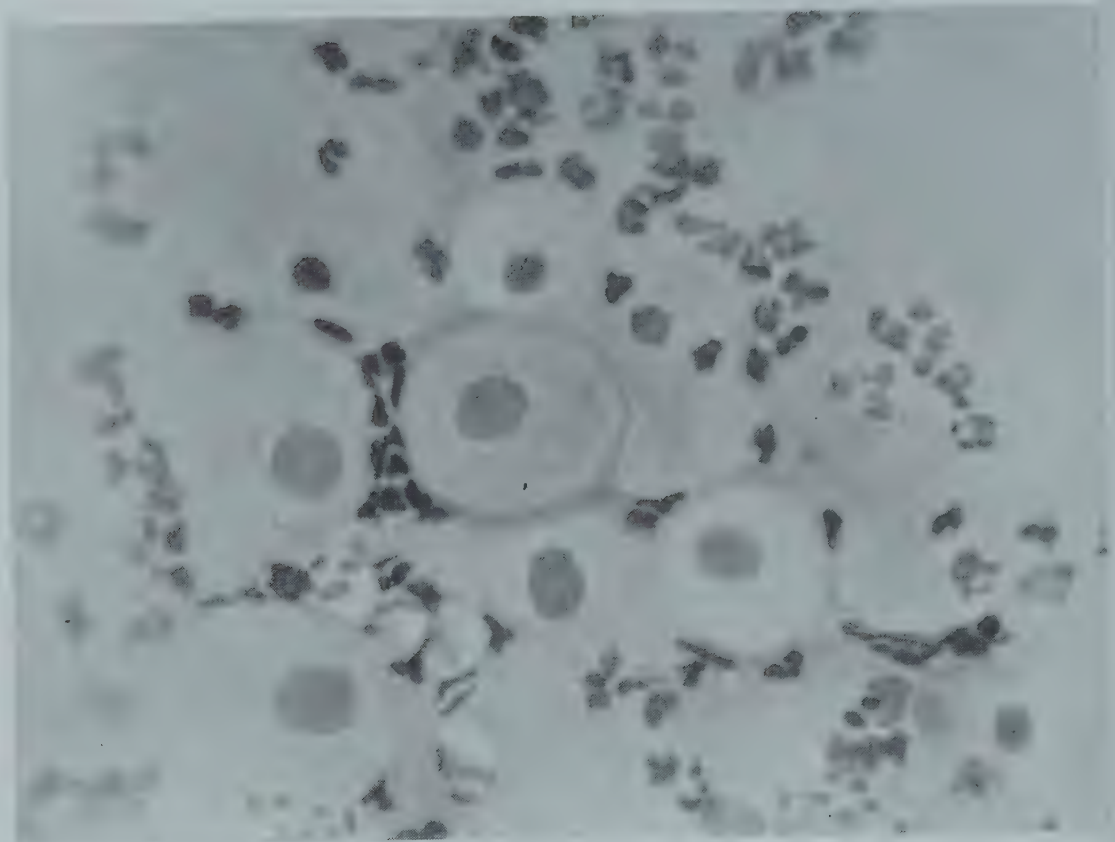


FIG. 27.—VAGINAL SMEAR. MENOPAUSE

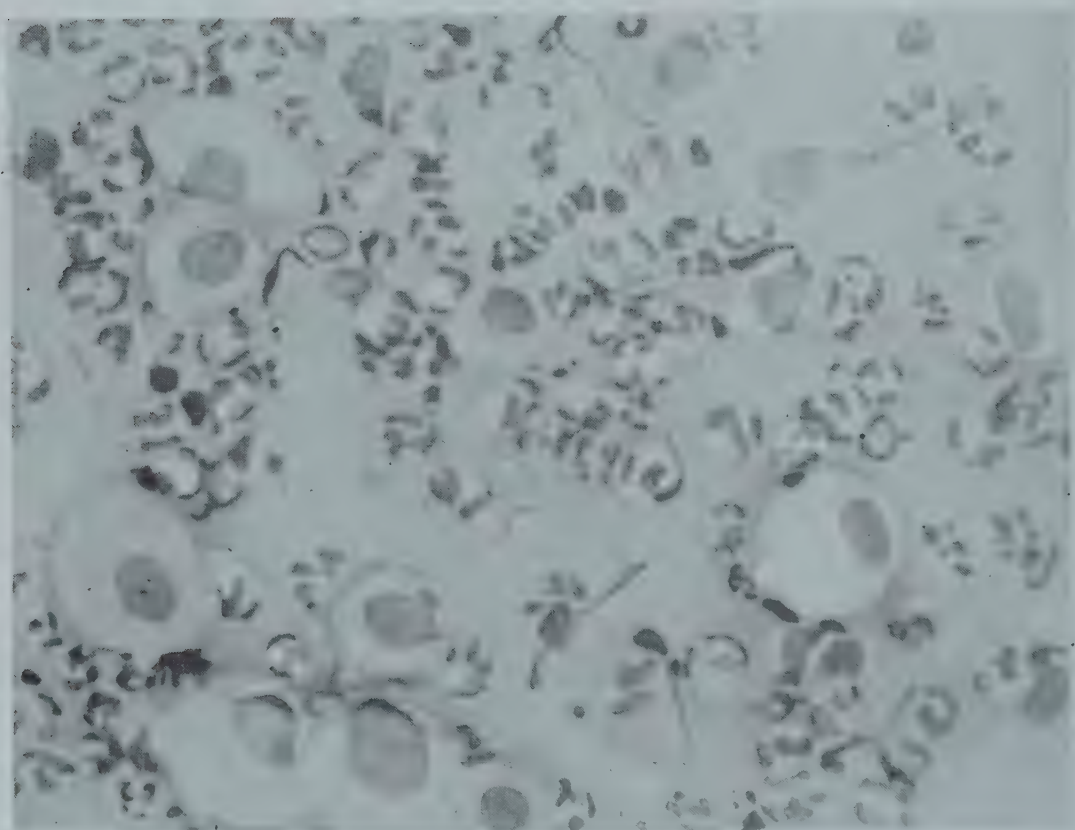


FIG. 28.—VAGINAL SMEAR. POST-MENOPAUSE



The abnormal cells appear either singly or in groups. Their nuclei are usually hyperchromatic to the point of staining sometimes entirely black. They frequently show signs of increased activity, evident by clumping of the chromatin, granularity and sometimes increase in the amount of nuclear material. Mitotic figures may occur but are extremely rare.

Often giant cells are found which are multinucleated, occasionally containing as many as a dozen nuclei. Nucleoli are frequently seen but are of no diagnostic value. The same applies to the appearance of vacuolated cytoplasm, which gives the cell a foaming appearance, and to the presence of histiocytes. Red blood cells or evidence of old bleeding is usually present. Many leucocytes, chiefly polymorphonuclears, are commonly found. They are clumped and scattered and many are seen engulfed by the cells.

Some fully cornified cells are usually present. Their significance as to oestrogenic activity has to await further investigation. Increased cornification is only rarely seen in women of post-menopausal age.

### *Undifferentiated Type*

The cells are smaller and more uniform in size and occur often in groups. The nuclei, which are hyperchromatic, show marked variation in size even to 100 per cent; the nuclei stand out against a homogeneous background of cytoplasm in which cellular borders are often indistinct. If single cells are encountered, an increase in nuclear size in relation to cytoplasm is the most important diagnostic aid. Adenocarcinoma of the cervix also shows cells of this undifferentiated type.

The diagnostic criteria for endometrial cancer are similar to that for undifferentiated cervical cancer. There is however less variation in size and shape of the nucleus and there is less difference in size from the normal nucleus as compared with nuclear changes occurring in cervical cancer. Occasionally, however, great variation in nuclear size may be encountered. Abnormal endometrial cells are usually found in clumps. The cells have unusually large hyperchromatic nuclei with very little cytoplasm. Large cells with an abundance of cytoplasm and large vesicular nuclei may occasionally be found.

A difficulty in the diagnosis of cancer cells is encountered when histiocytes, normal endometrial cells and atrophic cells of the basal layer of the vagina are present in the smear, thus leading to a false positive diagnosis. Meigs and co-workers [179, 180] describe these cells and their significance in the following terms:

1. *Foreign Body Giant Cells.*—Very large cells, sometimes containing as many as twenty nuclei with a tendency to peripheral arrangement. Occasionally, however, there may be so many nuclei that they occupy the whole cell. There is regularity in nuclear size with adequate amount of cytoplasm present.

2. *Small Single Histiocyte without Ingested Material.*—These cells often occur in groups and are then apt to be mistaken for undifferentiated cancer cells. They are smaller than these cells, however, have a foamy vacuolated cytoplasm, and nuclei uniform in size.

3. *Histiocytes.*—Large cell, with vacuoles containing ingested material, such as red blood-cells and leucocytes. These are easy to recognize and their presence does not lead to errors in diagnosis.

4. *Normal Endometrial Cells.*—These show hyperchromatic nuclei, little cytoplasm, and small variation in nuclear size.

5. *Atrophic Basal Cells.*—When a vaginal smear is composed entirely of basal cells, aberrant forms are often encountered, which are not easy to classify. The administration of stilboestrol 1 mg. daily for 10 days, however, will result in complete replacement of the basal cells by cornified cells and disappearance of the confusing atrophic cells.

*Malignant Giant Cells.*—The nuclei vary a great deal in size and stain much more deeply than those of the foreign body giant cells. They usually fill the entire cell, so that only a thin rim of cytoplasm remains visible.

*Undifferentiated Cancer Cell.*—The foamy vacuolated cytoplasm is absent; there is great variation in nuclear size; and the entire cell is larger than the histiocyte.

*Endometrial Cancer.*—Abnormal endometrial cells are usually found in clumps and there is greater, though little, variation in nuclear size.

### Preparation of Endocervical Smears

An ordinary cotton applicator is inserted about 1 inch into the cervical canal, twirled around a few times in one direction and is then rolled upon a slide. The slide should be placed at once for fixation into a



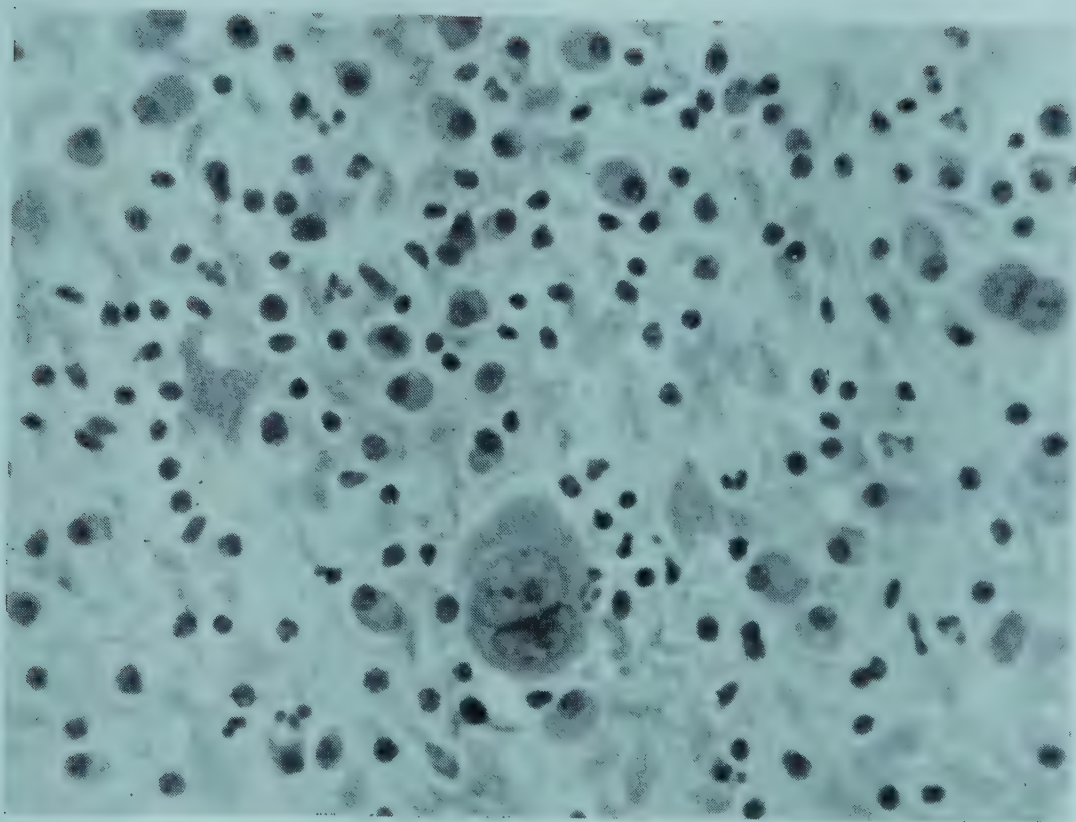


FIG. 29.—ENDOCERVICAL SMEAR OF 62-YEAR-OLD PATIENT

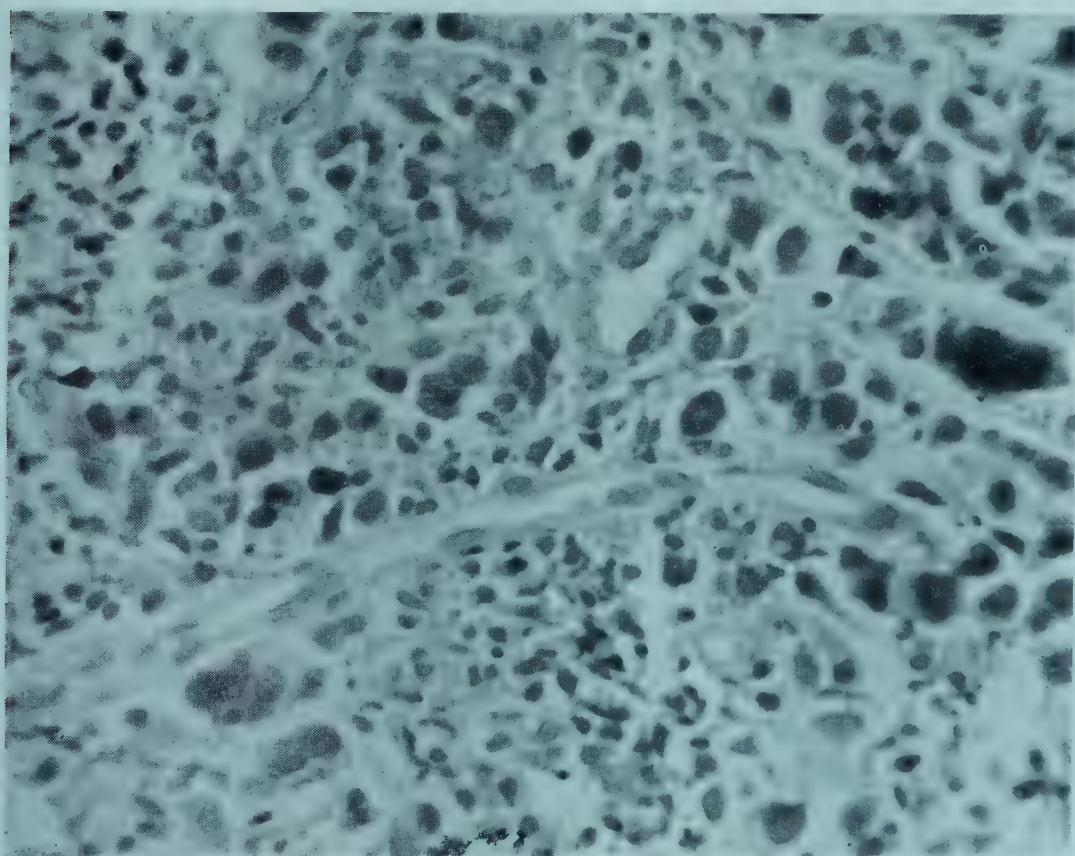


FIG. 30.—CERVICAL BIOPSY. ANAPLASTIC CARCINOMA



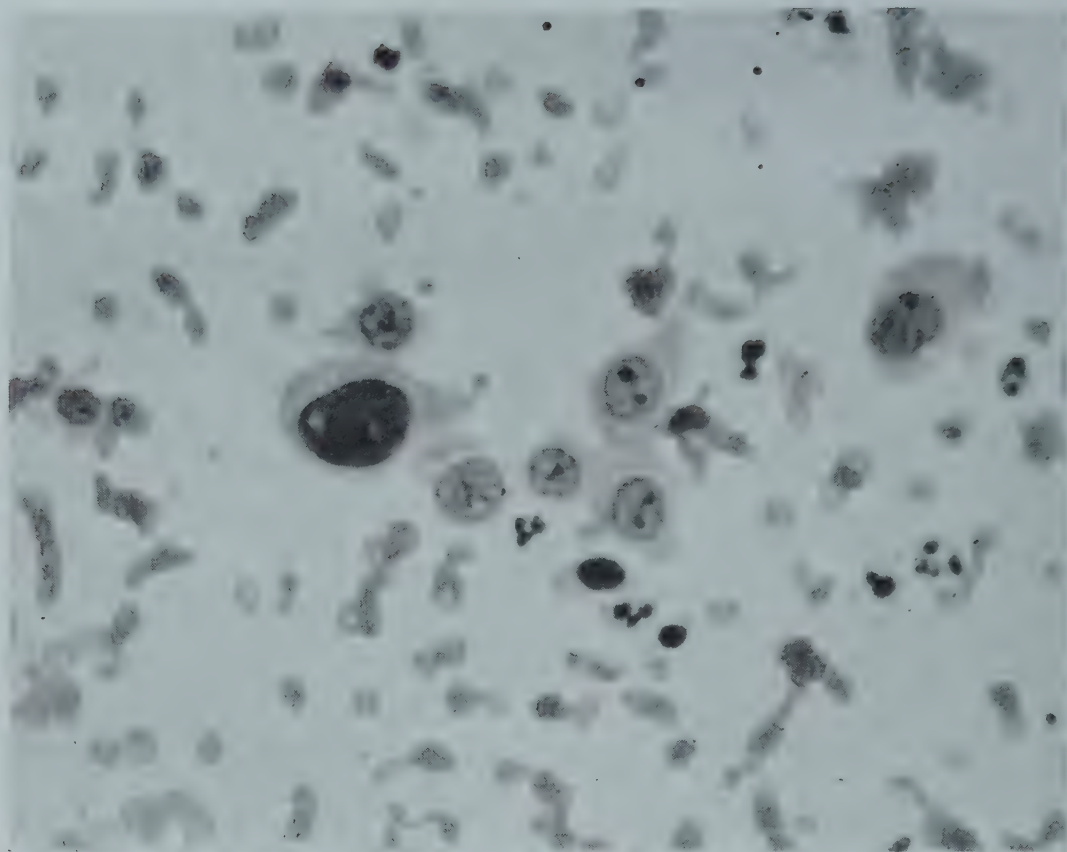


FIG. 31.—ENDOCERVICAL SMEAR OF 43-YEAR-OLD PATIENT

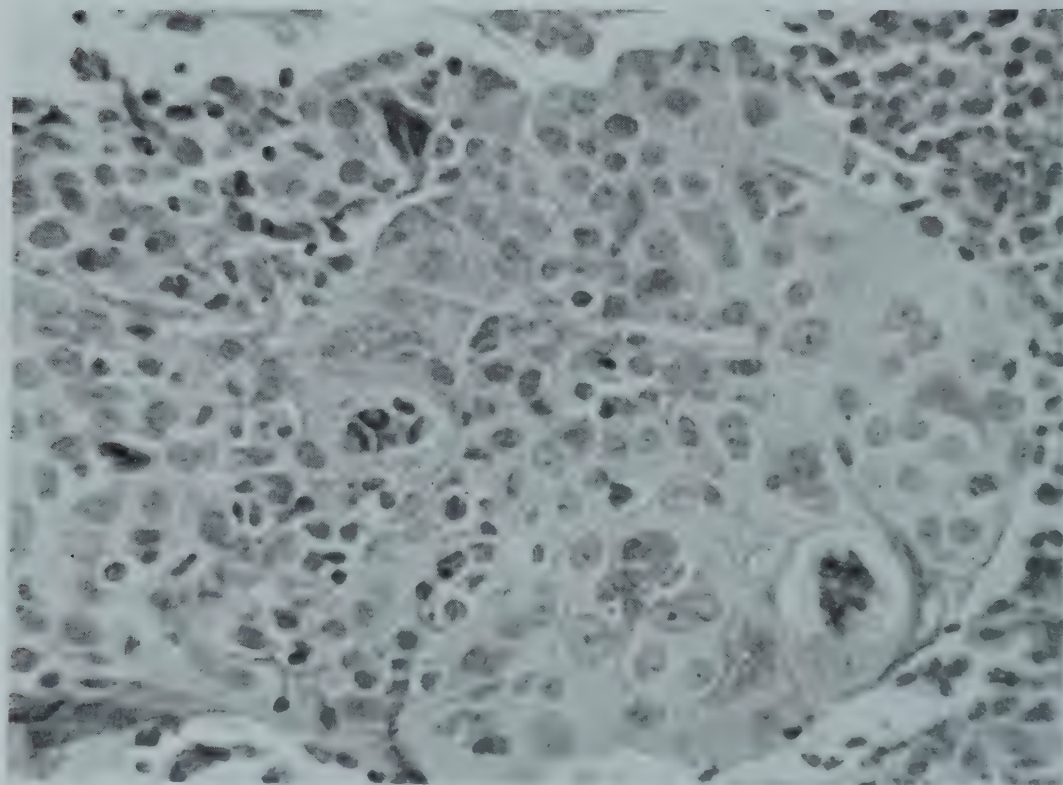


FIG. 32.—CERVICAL BIOPSY. RAPIDLY GROWING INFILTRATING SQUAMOUS CELL CARCINOMA

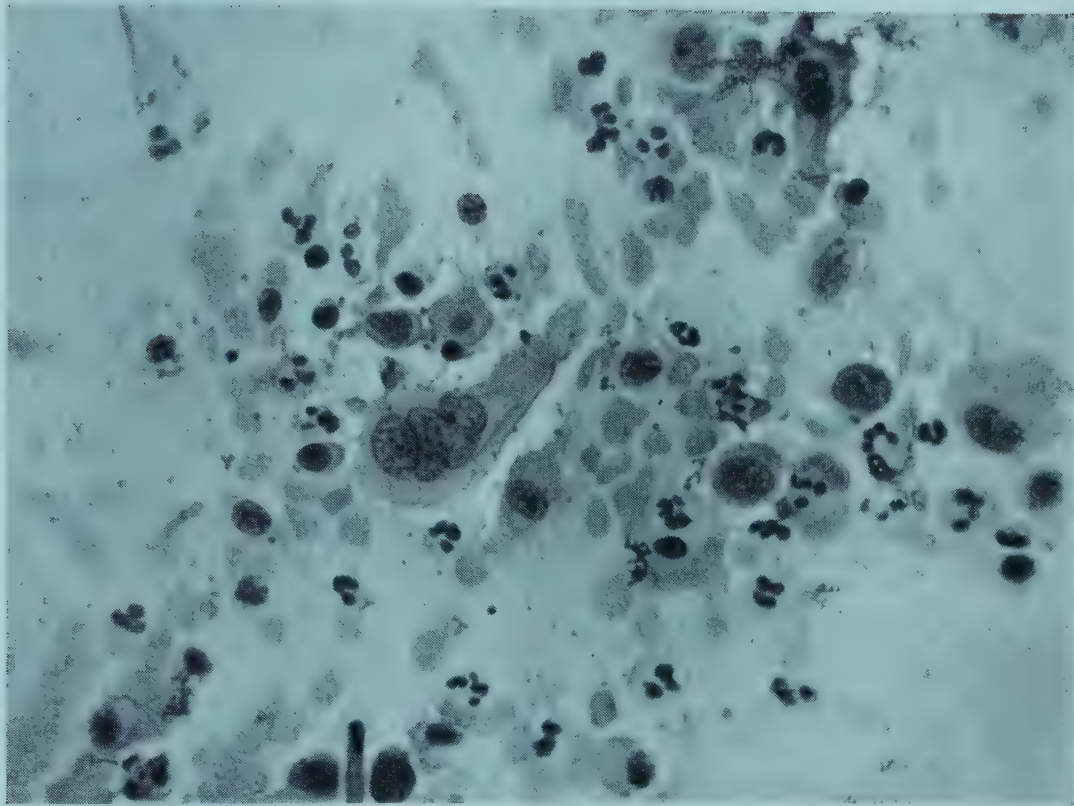


FIG. 33.—ENDOCERVICAL SMEAR OF 38-YEAR-OLD PATIENT

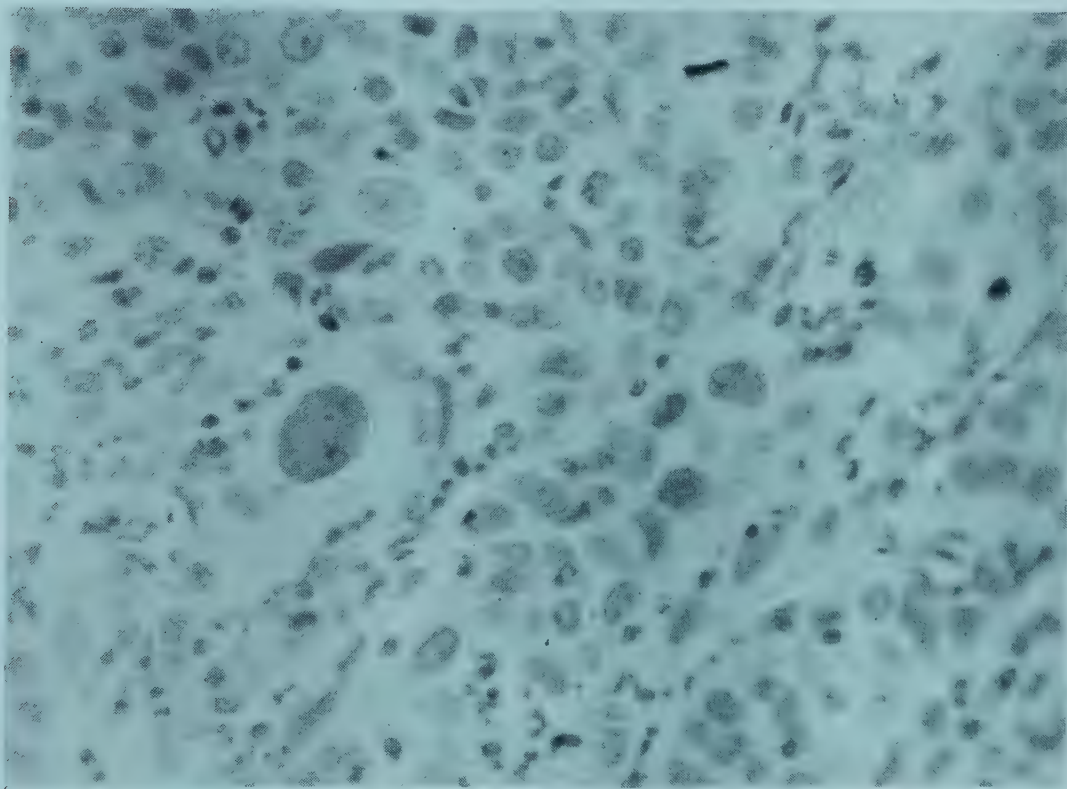


FIG. 34.—CERVICAL BIOPSY. RAPIDLY GROWING SQUAMOUS CELL CARCINOMA



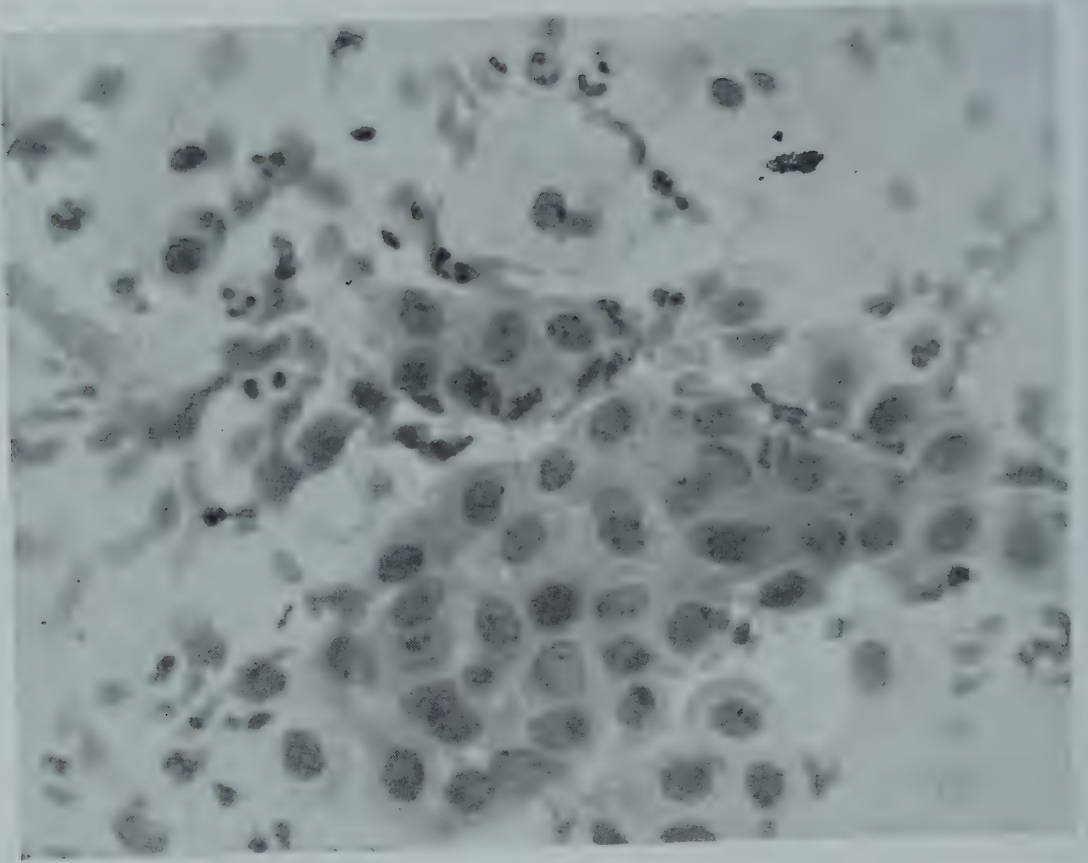


FIG. 35.—ENDOCERVICAL SMEAR OF 58-YEAR-OLD PATIENT

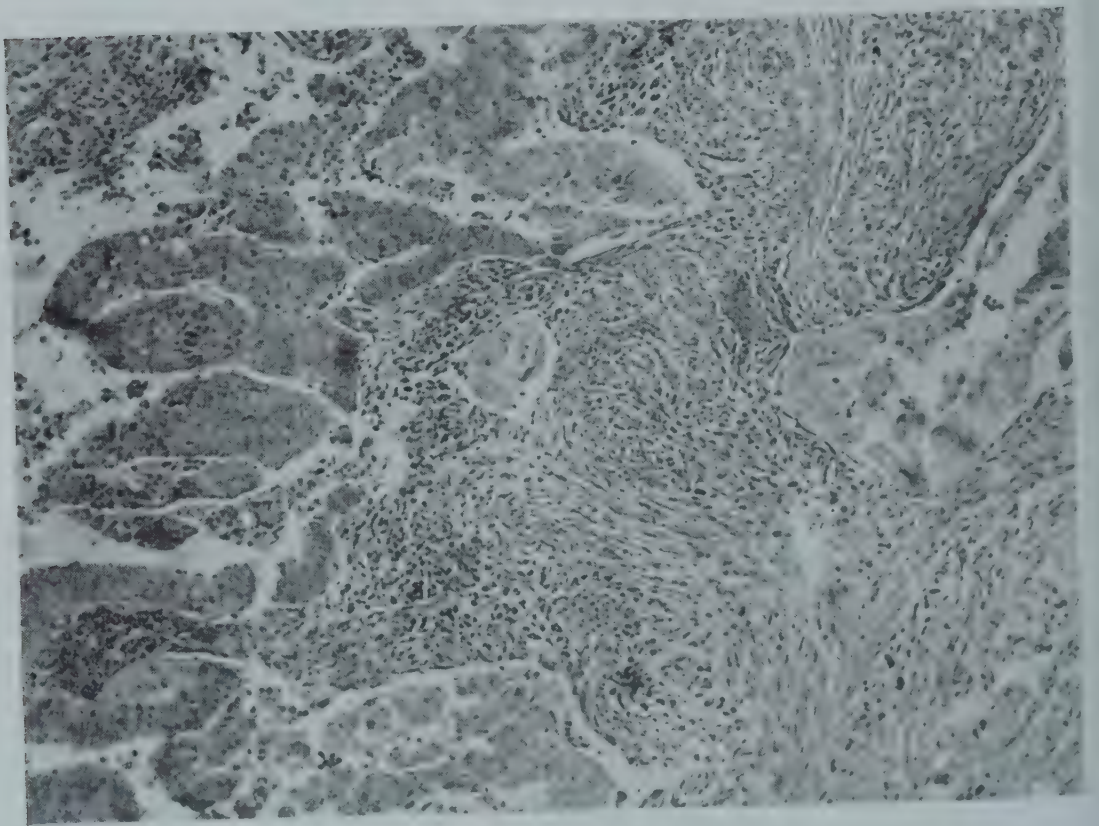


FIG. 36.—DIFFUSE ADENOCARCINOMA OF UTERUS



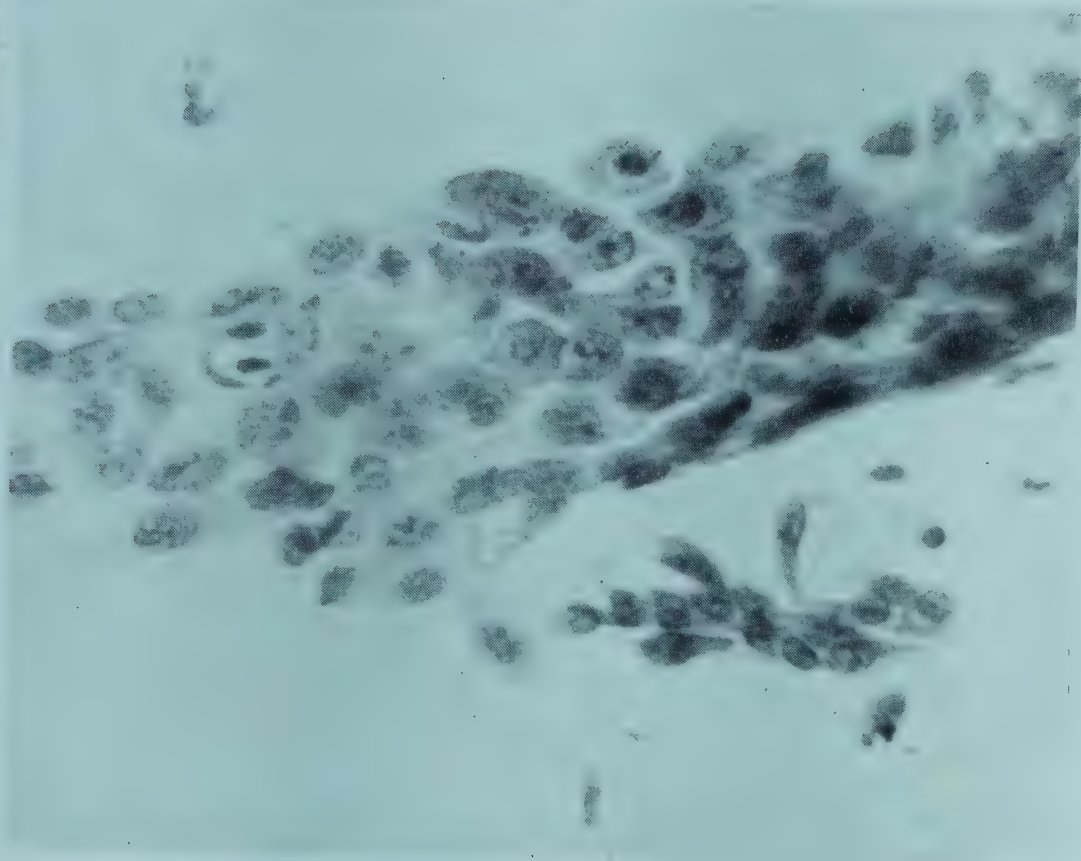


FIG. 37.—ENDOCERVICAL SMEAR OF 62-YEAR-OLD PATIENT

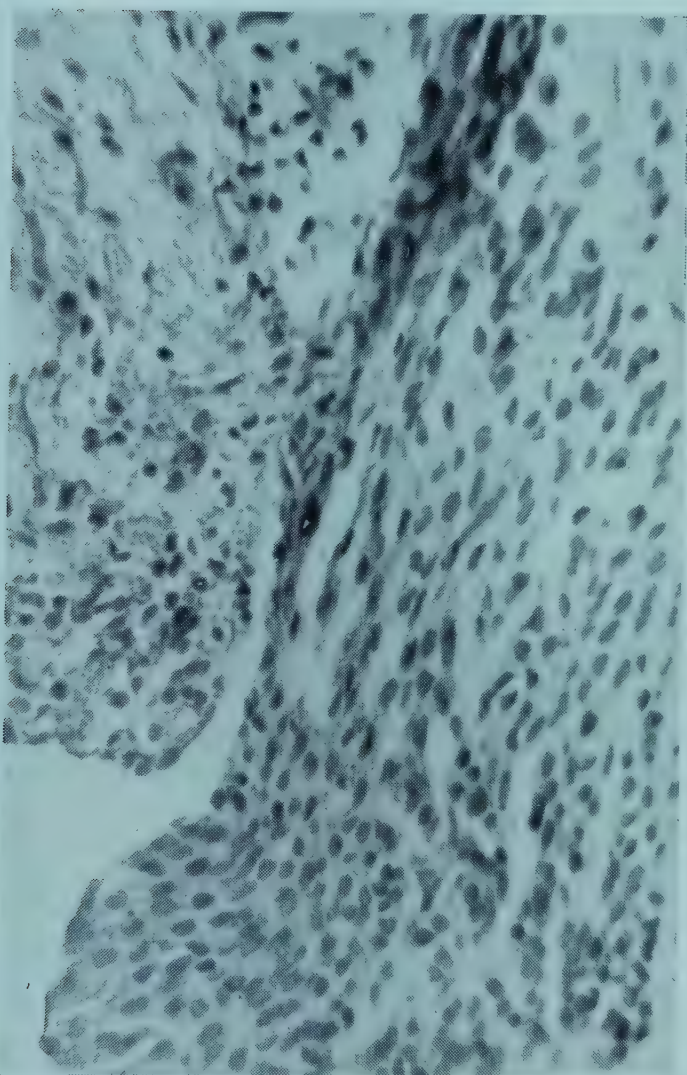


FIG. 38.—CERVICAL  
BIOPSY. PRE-INVASIVE  
CARCINOMA

solution of equal parts of ether and 95 per cent alcohol. It may remain there from 5 minutes to any length of time desired. If the slides are mailed, 1 or 2 drops of glycerin are released on the slide and it is then covered by another clean slide [211].



FIG. 39.—ENDOCERVICAL SMEAR OF 42-YEAR-OLD PATIENT

### *Methods of Staining*

#### *Papanicolaou's Stain*

The best stain available for the study of cancer cells is that of Papanicolaou. It affords the best transparency and greatest nuclear detail.

- i. After fixation the slide is carried through 80 per cent, 70 per cent and 50 per cent alcohol into distilled water.

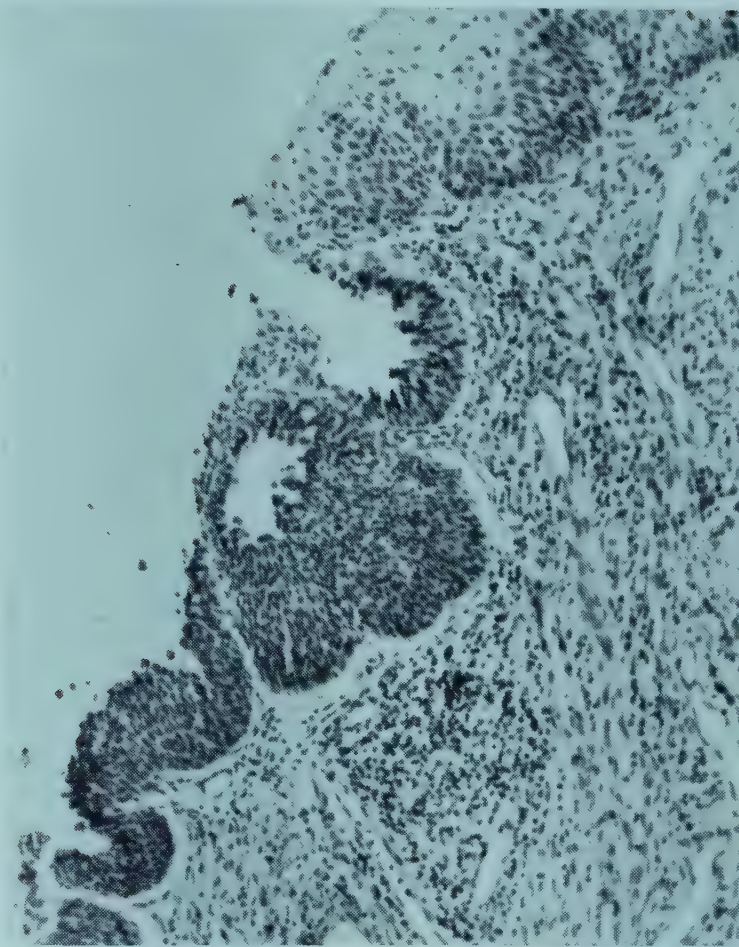


FIG. 40.—CERVICAL BIOPSY SHOWING PRE-INVASIVE  
SQUAMOUS CELL CARCINOMA

(Tissue sections of Figs. 30, 32, 34, 36, 38 and 40 by kind permission of Dr. Edgar R. Pund, Professor of Pathology, University of Georgia)

2. The smear is stained with Harris's haematoxylin\* for about 5–10 minutes and then washed in running water for at least 15 minutes.

\* HARRIS'S HAEMATOXYLIN

haematoxylin	.	.	.	.	.	1.0 gram
absolute alcohol	.	.	.	.	.	10.0 c.c.
potassium or ammonium alum	.	.	.	.	.	20.0 gram
mercuric oxide (yellow)	.	.	.	.	.	0.5 gram
distilled water	.	.	.	.	.	200.0 c.c.

*Method of Preparation*

1. Dissolve haematoxylin in the alcohol.
  2. Dissolve alum in water and boil.
  3. When alum solution is steaming, add the haematoxylin solution.
  4. Bring quickly to boil and add the mercuric oxide.
  5. When solution turns dark purple, remove from heat and cool rapidly.
- Add 4 c.c. of glacial acetic acid to every 100 c.c. of haematoxylin before use.



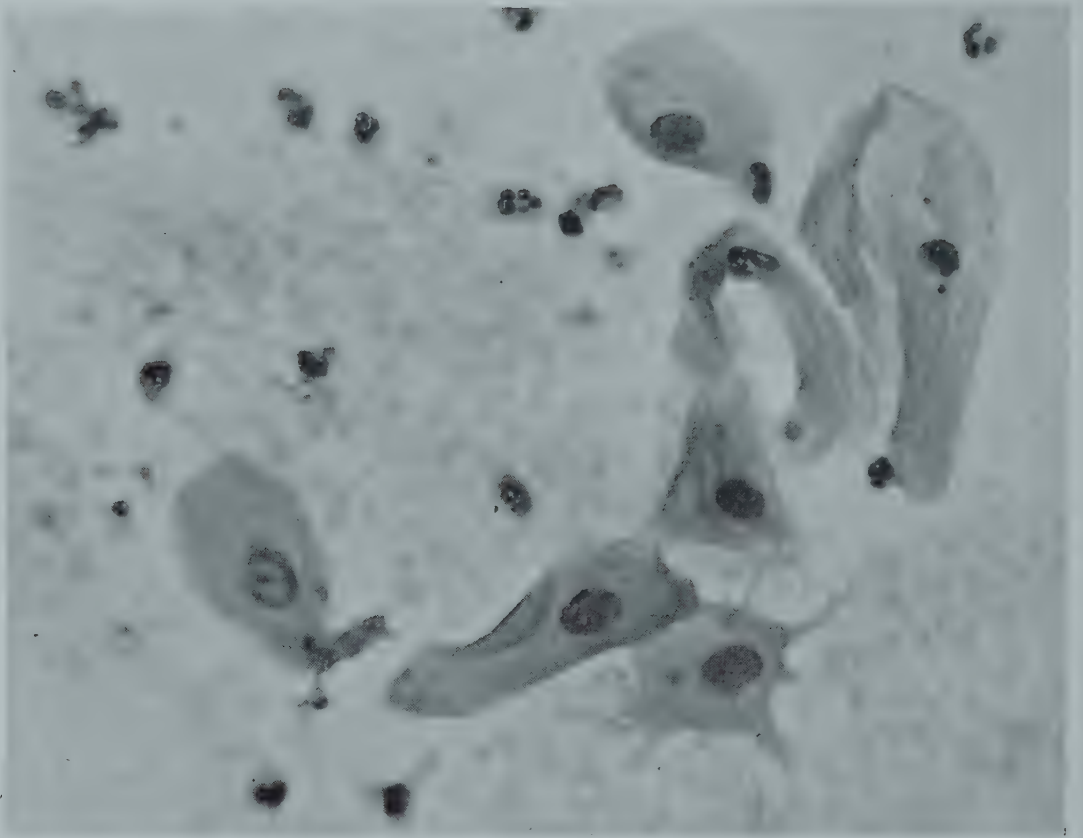


FIG. 41.—ENDOCERVICAL SMEAR SHOWING RADIATION CHANGES

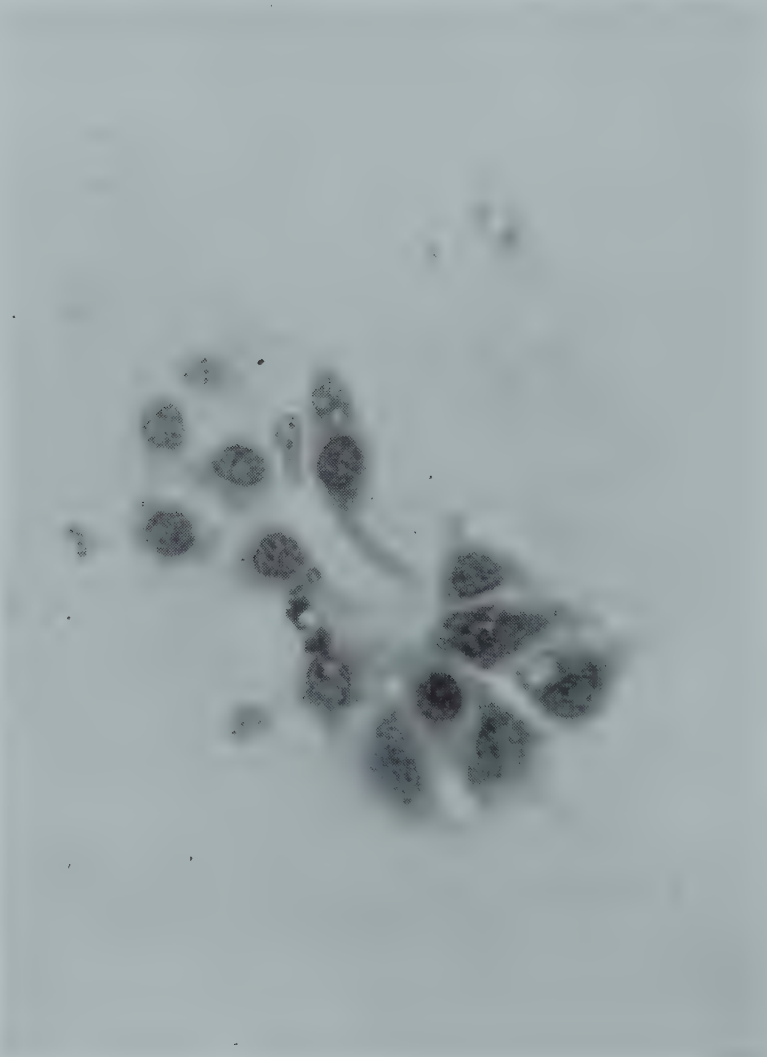


FIG. 42.—  
ENDOCERVICAL SMEAR  
SHOWING RADIATION  
CHANGES

3. It is then placed for 2 minutes in OG 6, i.e. 0.5 per cent solution of Orange G in 95 per cent alcohol (100 c.c.), 0.015 gm. Acid phosphotungstic.
4. After placing the slide for a few seconds in 95 per cent alcohol, it is stained for 3 minutes in solution EA 31\* [69].
5. It is then carried through 50 per cent, 70 per cent, 80 per cent, 95 per cent and absolute alcohol, cleared in xylol and mounted in balsam.

The cornified cells show a red to orange colour while the basal cells and other non-cornified cells stain green or blue-green.

### *Shorr's Stain*

Shorr recommends a rapid method for staining endocervical and vaginal smears with his single differential stain [70, 71, 81].

ethyl alcohol (50 per cent) . . . . .	100.0	c.c.
Biebrich Scarlet (water soluble) . . . . .	0.3	gram
orange G . . . . .	0.100	gram
aniline blue (water soluble). . . . .	0.025	gram
fast green FCF . . . . .	0.075	gram
phosphotungstic acid, c.p. . . . .	0.25	gram
phosphomolybdic acid, c.p. . . . .	0.25	gram
glacial acetic acid . . . . .	1.0	c.c.

After fixation in equal parts of ether and 95 per cent alcohol (1) the slide is placed for about 2 minutes in the staining solution; (2) it is then dipped 10 times in 80 per cent, 95 per cent and absolute alcohol; (3) the slide is dehydrated in xylol and mounted in balsam.

### **Papanicolaou and Shorr's Method [177]**

The following modification of Shorr's stain has proved more satisfactory in showing nuclear details.

1. It is essential that the swab should be fresh.

\* EA 31

light green SF yellowish, 0.5 per cent solution in 95 per cent alcohol . . . . .	50.0	c.c.
Bismarck Brown, 0.5 per cent solution in 95 per cent alcohol . . . . .	10.0	c.c.
eosin yellowish, 0.5 per cent solution in 95 per cent alcohol . . . . .	42.0	c.c.
acid phosphotungstic . . . . .	0.170	gram
lithium carbonate, saturated aqueous solution . . . . .	1 drop	

(A slight modification of this stain is commercially available under the trade name of EA 50.)

2. Roll swab over grease-free slide.
3. Fix while wet in equal parts (alcohol, 95 per cent., and ether for at least 5 minutes).
4. Take through the alcohols to water.
5. Stain in Harris' haematoxylin for 2 minutes.
6. Blue in tap water.
7. Differentiate in 0.5 per cent acetic acid in absolute alcohol.
8. Rinse in tap water for 10 minutes.
9. Stain in Shorr's stain for 10 minutes.
10. Rinse very rapidly in water. Take through the alcohols rapidly. Clear in xylol and mount.

### Results

- Cornified cells—orange-red.
- Non-cornified cells—green.

### Formulae

#### SHORR'S STAIN

50 per cent alcohol . . . . .	100.0	c.c.
water soluble Biebrich Scarlet . . . . .	0.5	gram
orange G . . . . .	0.25	gram
fast green. . . . .	0.25	gram
phosphotungstic acid . . . . .	0.075	gram
phosphomolybdic acid . . . . .	0.5	c.c.
glacial acetic acid . . . . .	1.0	c.c.

#### Modified Best's Carmine Method [178]

1. Fix the smear by drying in air.
2. Stain with haematoxylin for 5 minutes.
3. Rinse in cold water.
4. Stain with carmine solution for 15 minutes.
5. Differentiate in:

absolute ethyl alcohol . . . . .	16	c.c.
absolute methyl alcohol . . . . .	8	c.c.
distilled water . . . . .	20	c.c.

Immerse 4-5 times.

6. Absolute alcohol—2 immersions.
7. Xylol—2 immersions.
8. Mount with balsam.

The glycogen appears as deep red granules in the cytoplasm. In some cells, the granules are coarse, while in others they are so fine that their cytoplasm is diffusely pink. The nuclei stain blue. This



stain also reveals distinctly the morphological characteristics of the cellular elements of the smear.

### Mack's Test for Cellular Glycogen

A quick simple quantitative test for cellular glycogen using the iodine vapour technique has been described by Mack [72, 73].

The technique is as follows:—

1. *Preparation of Smears.*—A cotton applicator is inserted into the vagina and twirled lightly (one complete rotation) against the vaginal wall. The cotton end of the applicator is then rolled lengthwise over the surface of a clean glass slide. By rolling, rather than rubbing, a uniformly thin film of cells results, with minimal clumping and cell distortion. The film dries almost immediately and may be stained at once.
2. *Staining of Smear.*—Staining is accomplished by laying the slide, face down, over a shallow dish containing a small amount of Lugol's solution. Iodine vapours which arise insensibly from the solution suffice to stain the glycogen-containing cells in two or three minutes. Microscopic examination may be carried out immediately. Although such stains fade in 24–28 hours, re-staining (by the same method) may be carried out repeatedly if later examinations are desired.

According to the content of glycogen present and its intracellular distribution, the stain is graded into four degrees [73]:

1. Complete glycopaenia. Smears of this type contain only small yellow cells of varying size and shape and large amounts of amorphous cellular debris. In extreme grades there is marked paucity of pithelial elements.
2. This grade of smear is marked by a greater abundance of epithelial elements than in grade 1. Iodine-vapour staining depicts glycogen in irregular brown deposits at the cell margins or scattered irregularly throughout the cytoplasm ("mottled cells"). Diffusely-stained brown cells, usually of the small, round variety (deep cells; atrophy cells) may also be present in small numbers. Many glycopaenic yellow cells are also found.
3. A further increase in cell numbers is evident in this grade compared to the preceding. The cells are larger and more

regular. The diffusely-stained cytoplasm has a rich brown colour. Non-iodophilic yellow cells are also present in abundance.

4. This grade of smear is easily recognized by the presence, almost exclusively, of large, flat, deeply-stained brown iodophilic cells, present singly or in large clumps. Grade 4 represents maximal oestrogenic effect and corresponds to the smear of the normal follicular phase.

### BODY TEMPERATURE

Body temperature closely follows hormonal changes during the normal cycle as well as in abnormal conditions [121-7]. The difference between rectal, vaginal and oral temperature is one of degree only. The charted graph in each case represents the same pattern of curve, decrease of temperature denoting oestrogen activity and rise of temperature indicating the presence of progestogen. The administration of each of the hormones has been shown to induce identical changes.

The fluctuations of temperature in the normal cycle range between  $0.3^{\circ}$  and  $1^{\circ}$  F. [122] and the extreme levels during the whole cycle between  $0.9^{\circ}$  and  $1.6^{\circ}$  F. [123]. The author found the difference between the lowest point of the follicular and highest point of the luteal phase to be about  $1.8^{\circ}$  F. In cases of ovarian deficiency the difference is smaller, decreasing with the degree of deficiency [127] (see Fig. 43).

### ORAL AND VAGINAL TEMPERATURE TAKEN SIMULTANEOUSLY

Oestrogen and progestogen produce a greater increase or lowering of the oral than of the vaginal temperature, oestrogen being more potent in this respect than progestogen. When progestogen acts in the presence of oestrogen, however, the temperature increase is greater in the vaginal curve owing to the more depressing effect of oestrogen on the oral temperature [127] (see Fig. 44). Marked rises of temperature occur after taking alcoholic drinks [126].

### Normally Menstruating Women

#### *Pre-menstrual Phase*

Fall of temperature about 1-2 days before the period is due.

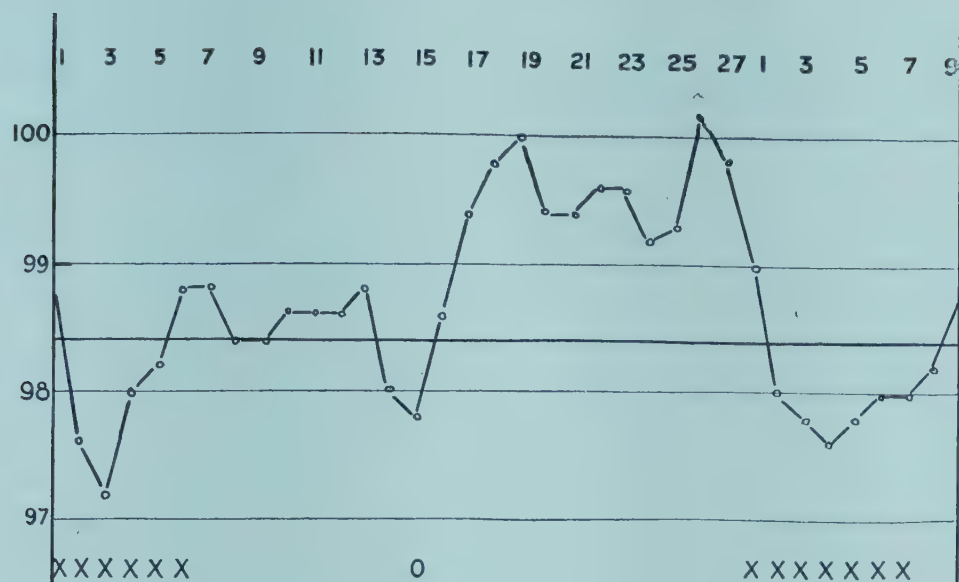


Fig 43

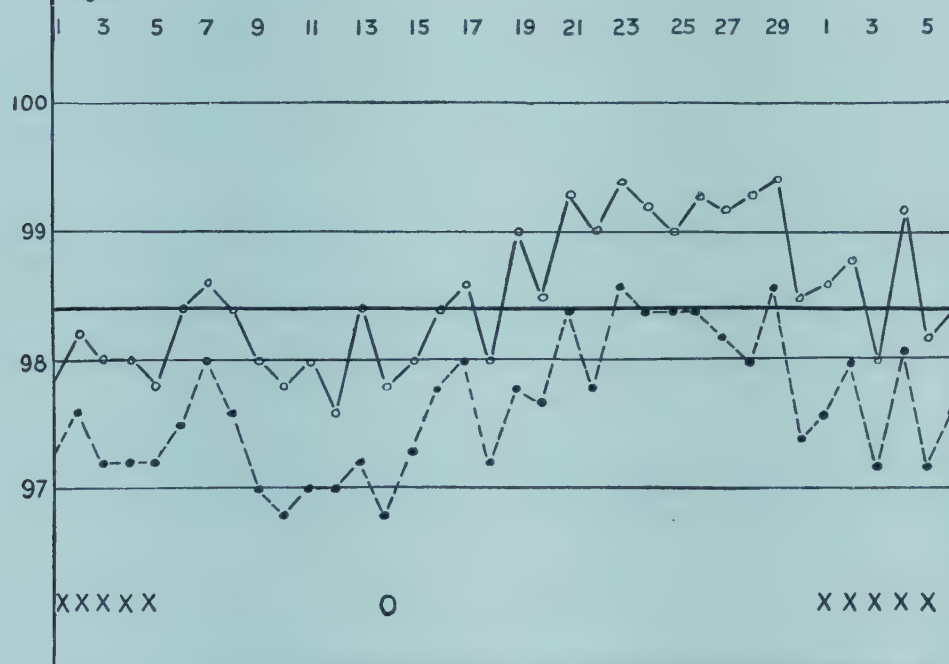


Fig 44

FIG. 43.—VARIATIONS IN VAGINAL TEMPERATURE

FIG. 44.—VARIATIONS IN VAGINAL AND ORAL TEMPERATURE

(X = menses; O = ovulation)

### Menstrual Phase

Further decrease of temperature which may reach a level as low as 97° F. or lower, followed by a rise to about 98°–99° F.



*Follicular Phase*

Low fluctuating temperature of about  $98.4^{\circ}$ – $99^{\circ}$  F. or lower.

*Ovulatory Phase*

Sudden fall of temperature to about  $97^{\circ}$  F. or lower, usually on the fourteenth day before the onset of menstruation, followed by a steep rise. Frequently no fall precedes the sudden rise of temperature.

*Luteal Phase*

Sustained elevation of temperature reaching  $100^{\circ}$  F. or higher, continuing until 1 or 2 days before menstruation.

*Mid-luteal Phase*

A slight fall of temperature takes place at this phase, corresponding probably to the peak of oestrogen production.

*Pregnancy.*

Following the onset of pregnancy the temperature curve shows a sustained elevation [121, 126].

*Abnormal Conditions*

Endocrine disorders causing changes of sex-hormone levels are reflected in the temperature charts, which are thus of diagnostic value.

*Hyper-oestrinism*

A hyper-oestrogenic state due to hyper- or polyfollicular activity is associated with a persistently low curve, either fluctuating or constant [121, 127].

*Hypo-oestrinism*

In cases of oestrogen deficiency the temperature curve tends to be on a higher level and less fluctuating [121, 127].

*Persistent Luteal Activity*

This condition is invariably associated with a high temperature curve, depending for its fluctuations on the amount of oestrogen present [127].

*Sterility*

The temperature curve is valuable in the treatment of sub-fertility and sterility, offering as it does a simple device for detecting the onset of ovulation [121–7].

## BASAL METABOLISM

The "basal metabolism" refers to the minimal heat production of the body measured by its oxygen consumption under conditions of complete physical and mental rest.

The most commonly used apparatus for the determination of oxygen consumption makes use of the closed or spirometric method. It consists of an oxygen reservoir or spirometer and a chamber containing soda lime which removes the carbon dioxide. Respiration causes an up-and-down movement of the spirometer bell suspended on water which records the respiratory movements on a uniformly revolving drum. Inspirations produce the up-strokes, and expirations the down-strokes.

The patient should retire early the night before the test. No food may be taken after 8 p.m. and the test should be conducted on the following morning from 12 to 16 hours after the ingestion of food. Records are made of the patient's height, weight, age, sex, pulse rate and body temperature. The temperature of the air in the spirometer and the barometric pressure are also recorded. To carry out the test, a soft rubber mouthpiece is fitted closely between the patient's teeth and lips, and the nasal passages are shut off by an adjustable rubber clamp. The test proceeds for 5 or more minutes.

The figure representing the oxygen consumption per minute, obtained after a correction for the temperature and barometric pressure, is recorded as a percentage of the standard normal heat production (calories) per square meter of body surface and is known as the basal metabolic rate (B.M.R.). The basal metabolic rate is considered to be normal if the values obtained are between  $-10$  and  $+15$  per cent.

The tracing on the recording spirometer indicates whether breathing was automatic or whether some inhibition from higher cerebral centres was superimposed on the basic rhythm. When breathing is automatic, the spirogram has a regular pattern with little variation in the rate, depth or volume of ventilation. There is a tendency to pause at the end of each expiration, and the ability of the chest to return to the same resting position at the end of successive breaths is shown by an even apposition of the expiratory points to the "base line" throughout the record. If the subject is unable to relinquish conscious control of his breathing and so allow it to run autonomically, evidences of inhibition are clearly revealed in the respiratory tracing. There are abrupt changes in the rate, depth and volume of ventilation. There is a tendency to hurry the launching of each new

inspiratory effort, so that the normal expiratory pause is modified or even entirely absent. The superimposed control is not nearly so efficient in returning the chest to the same position at the end of successive breaths as is the normal mechanism of muscular relaxation of the thorax during expiration. This irregularity is recorded on the spirogram by abrupt, and often very large, deviation of expiratory points from the "base line" [128].

The B.M.R. of women is on an average about 7.5 per cent lower than that of men of the same age. There is an increase of metabolism during the pre-menstrual week averaging from 2.7 per cent to 3.7 per cent, and a slight fall during menstruation [129, 130].

#### CONDITIONS AFFECTING B.M.R.

Duncan summarizes as follows a number of pathological conditions which alter the basal metabolic rate [131]:

##### *Increased B.M.R.*

Hyperthyroidism  
Adenomata of thyroid gland  
Malignancy of thyroid gland  
Polycythaemia  
Severe anaemia  
Leukaemia  
Diabetes insipidus  
Advanced cardiac decompensation  
Drug poison (dinitrophenol)  
Diabetes  
Pseudo-dwarfism.

##### *Decreased B.M.R.*

Myxoedema  
Cretinism  
Thyroiditis (Riedel's disease)  
Nephrosis  
Hypopituitarism  
Simmonds's disease  
Addison's disease  
Undernutrition (starvation; anorexia nervosa)  
Shock.

#### CLINICAL SIGNIFICANCE OF B.M.R.

The following estimate of the value of basal metabolic rate determinations in clinical practice is summarized by Duncan [131] from Means [132].

1. Patients with an outspoken clinical picture of hyperthyroidism invariably show increased metabolism, and those with definite clinical pictures of hypothyroidism invariably show decreased metabolism. Those with goitres, but no signs or symptoms of abnormal function, for the most part show normal metabolism.

2. Patients with atypical or incomplete clinical evidence of abnormal thyroid function may show normal or abnormal metabolism. The majority show normal metabolism.

3. By inference from the indirect evidence we believe that in these borderline thyroid cases, provided that in the first place a true basal rate is secured and, provided that certain well recognised cases for increased



metabolism, such as fever, acromegaly, leukaemia and severe anaemia are excluded, the finding of an increased basal metabolic rate is strongly presumptive evidence of hyperthyroidism. In a similar way, provided that such conditions as starvation, hypopituitarism and hyposuprarenalism are excluded, a low metabolic rate is strongly presumptive evidence of hypothyroidism.

4. To that extent, then, the metabolism test is distinctly useful in differential diagnosis. Like all other laboratory tests it should be interpreted only with due regard to all other clinical and laboratory findings, and with due regard for its limitations and pitfalls.

#### ASPIRATION BIOPSY METHOD FOR THE EVALUATION OF THYROID FUNCTION [133]

A No. 18 or a No. 16 gauge 2-inch intravenous needle and an ordinary 20-c.c. syringe are used. It is necessary for the needle to be sharp and the syringe to be fairly new so that the barrel and piston do not fit loosely. Tissues obtained are placed in 5 per cent formalin and are treated as regular paraffin sections to ensure uniformity of results. A sedative may be given to the patient 20 minutes before the aspiration. The patient should be lying flat with the neck slightly hyperextended. The gland is palpated and a small area in the midline of the neck close to the gland is prepared aseptically and a wheal is raised in the skin with 1 per cent procaine. Some of the solution is injected continuously along the line of the intended puncture down to the gland. A small nick is made through the skin with a bistoury-pointed scalpel (No. 11 B.P. blade) with the instrument held at right angles to the skin surface. The puncture of the skin facilitates insertion of the needle and prevents its becoming plugged with surface epithelium. The needle with the syringe attached is introduced tilted at about 30 degrees to the sagittal plane. It is advanced slowly through the superficial tissues until the point is felt to enter one or other of the lateral lobes, whichever seems the more accessible. This technique will avoid the trachea, and the other important structures.

The extreme care with which this aspiration biopsy must be done is apparent from the detailed description of the procedure given by the authors.

Microscopic examination of the aspiration specimens shows only small particles of the gland in a large amount of blood. Closer examination under the oil-immersion lens reveals that these gland particles consist entirely of acinar cells, arranged in acini and portions of acini from which the colloid material is entirely gone. Apparently,

the force of the aspiration is chiefly exercised on the cells, and the colloid is squeezed out and left behind. If only single cells are left, the acinar cells appear to be intact and undistorted in their acini and remnants of acini.

Abel used the criterion that an average cell height of over 5.8 units indicated toxicity in nodular goitres, a height of over 5 units indicated toxicity in diffuse goitres and a height of less than 5 units indicated a normal gland. Each of his units equalled 1.4 microns, which gives the following standards of reference:

1. An average cell height of over 8.1 microns indicates toxicity in nodular goitres.
2. An average cell height of over 7 microns indicates toxicity in diffuse goitres.
3. An average cell height under 7 microns indicates a normal gland. These standards for cell heights were used as a criterion for diagnosis. The measurement of the thyroid acinar cells as described by Abel was performed with a micrometer scale fitted into the eyepiece of the microscope, which was used on an oil-immersed objective. In all cases the diameter of a cell was measured at a point crossing the nucleus, and on a line along the radius of an acinus. The cells were measured in blocks of 100, from 100 separate acini chosen at random where the scale happened to fall as the slide was systematically moved across the stage. The only provisos were that the acini should be open and the cell boundaries distinguishable.

## OVULATION

The time of ovulation is subject to individual variations, and may differ in various cycles. According to experimental evidence, ovulation occurs about 15 days before the expected menstruation, regardless of the length of the cycle. In a series of 118 laparotomies performed on women with variable cycle length, Ogino [150] found only unruptured graafian follicles in those operated upon 16 days before the expected flow. In those operated upon only 12 days before the estimated date, however, he found definite evidence of ovulation. Ovulation must therefore have occurred in Ogino's patients between the 12th and 16th days before menstruation, irrespective of the cycle length. The assumption that ovulation does not occur earlier than 15 days before the next period, regardless of the length of the

menstrual cycle, is now widely employed both for contraceptive purposes (the so-called "natural" method) and for the treatment of subfertility.

It has been suggested, however, that ovulation may occur more than once in a single menstrual cycle, and that even in the human it may be evoked prematurely by coitus [151, 152]. This view is supported by clinical records of pregnancy following isolated coitus during any phase of the menstrual cycle, even during menstruation [153]. The explanation offered is that great sexual excitement during intercourse may, in some women, evoke the production or release of a sufficient amount of gonadotrophic hormone to cause untimely ovulation [154]; or that the increased production of adrenalin during intercourse may cause rupture of the follicle [155]. The view that coitus or other extraneous stimuli may induce follicular rupture finds some support in the observation that conception may follow dilatation and curettage in some cases of functional sterility. It is tempting to explain such cases by assuming that mechanical irritation of the cervix induces ovulation through the pituitary, in the same way as a similar procedure produces this effect in the rabbit. Birnberg's report [156] that 23 out of 30 women showed a rise in gonadotrophic secretion within 30 hours after cervical dilatation, and that 3 of the women who were sterile conceived shortly afterwards, is of great interest in this connection. Further support is afforded by the fact that pregnancy sometimes follows a negative tubal insufflation [145] or salpingography [157].

#### METHODS EMPLOYED TO ASCERTAIN THE TIME OF OVULATION

The chief methods for detecting the time of ovulation are:

1. Microscopic examination of the endometrium.
2. Estimation of pregnanediol excretion.
3. Study of vaginal smears.
4. Study of basal temperature graphs.
5. Estimation of oestrogen and gonadotrophin in the blood.
6. Study of uterine reaction to pituitrin.
7. Electrometric procedures.

#### **1. Endometrial Biopsies**

The uterine mucosa accurately reflects events in the ovary. Early secretory changes in the endometrium normally follow follicular rupture and coincide with corpus luteum formation. This gives an



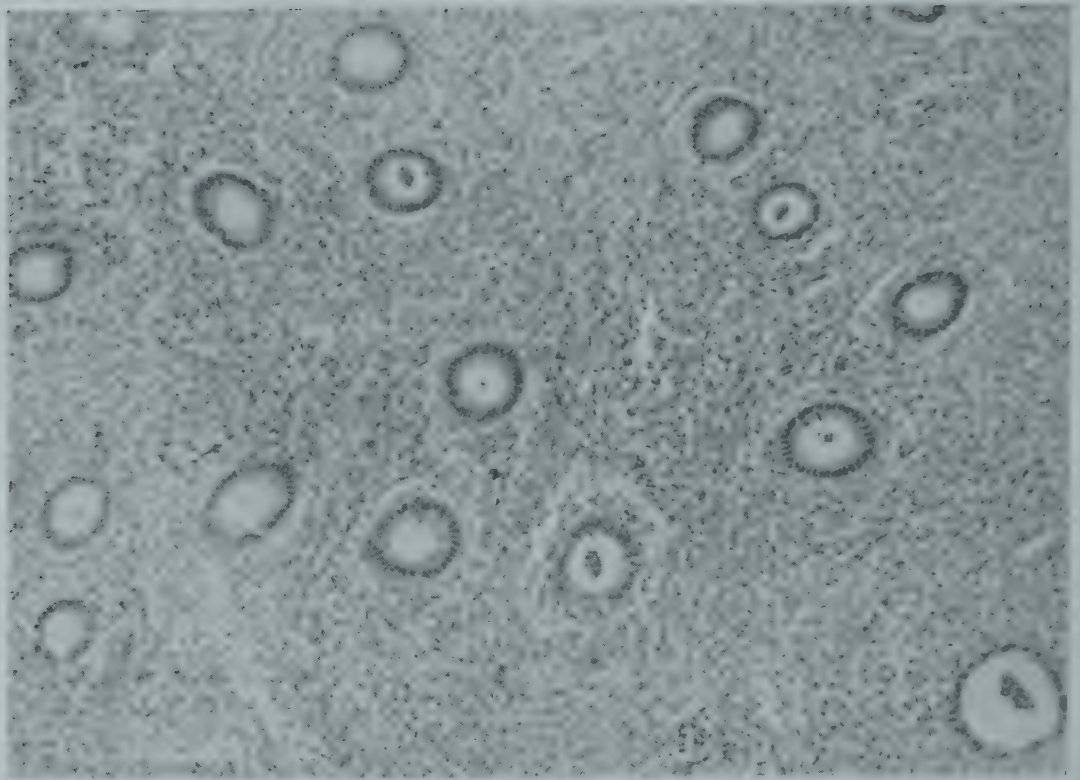


FIG. 45.—ENDOMETRIUM IN FOLLICULAR PHASE

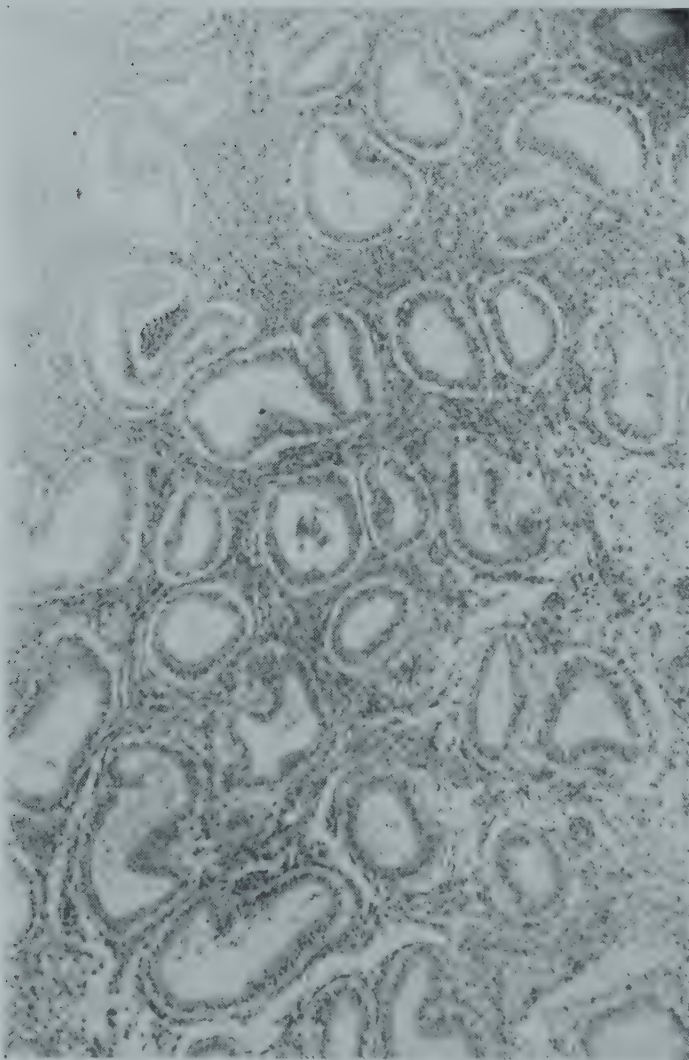


FIG. 46.—  
ENDOMETRIUM IN  
PRESECRETORY PHASE

*(Figs. 45-48 by kind permission of  
Dr. Edgar R. Pund, Professor of  
Pathology, University of Georgia)*

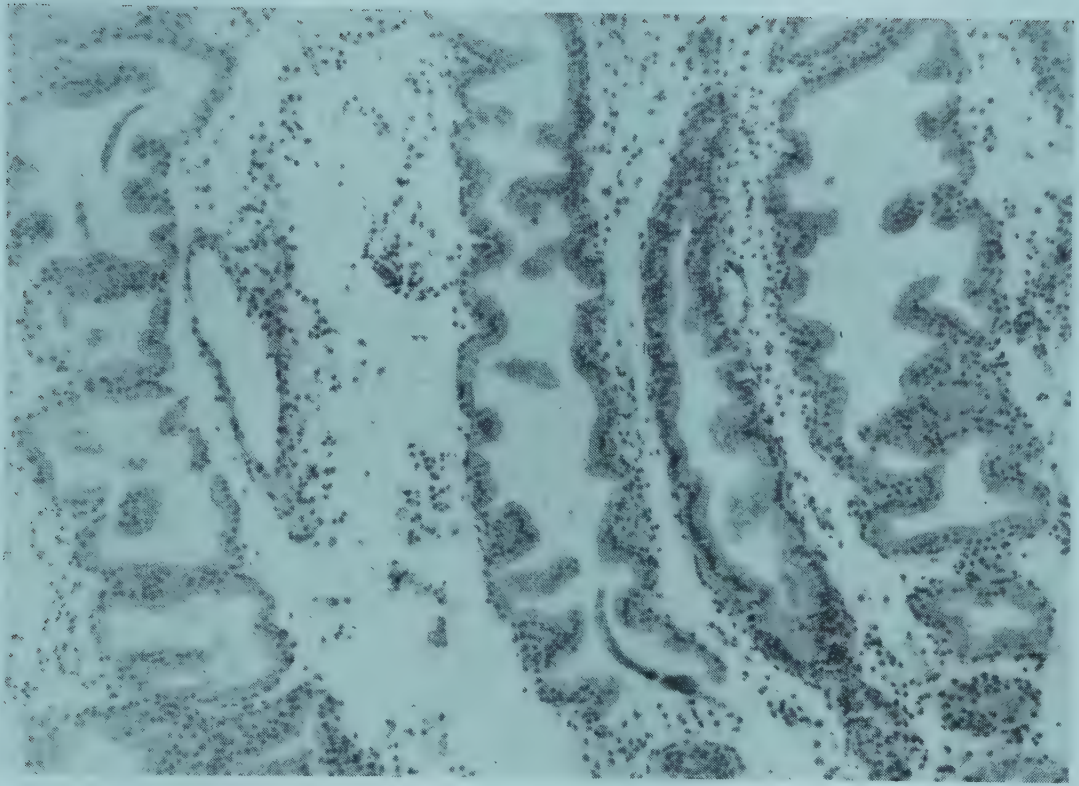


FIG. 47.—ENDOMETRIUM IN SECRETORY PHASE

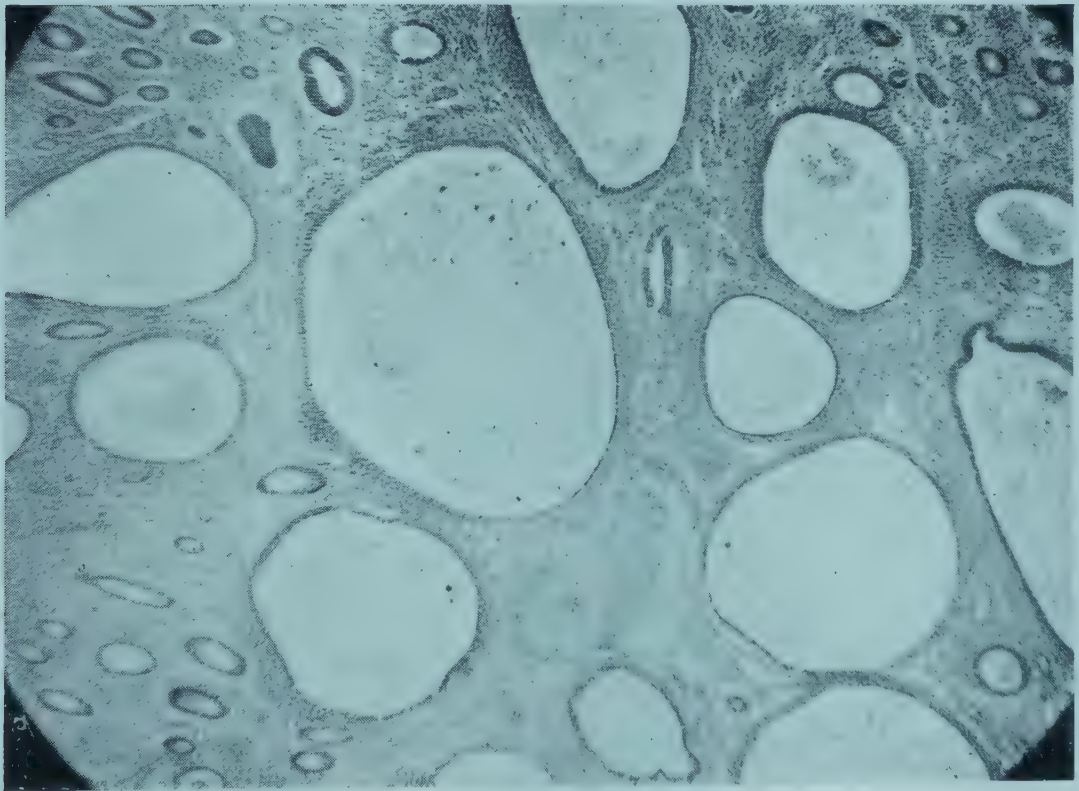


FIG. 48.—ENDOMETRIUM SHOWING CYSTIC GLANDULAR HYPERPLASIA



approximate idea of when ovulation occurred. However, according to Zondek [158] secretory changes in the endometrium may occur without rupture of the follicle. Westman and Zondek [159, 160], after removal of the ovarian tissue from the mature rabbit, leaving only one follicle, from which the ovum was extirpated, found that prolan injections caused transformation of this empty follicle into a typical corpus luteum. They attributed this to the transformation of the granulosa cells into lutein cells, following the administration of gonadotrophic hormone. These lutein cells produced progesterin, as shown by the progestational transformation of the uterine mucosa. A further difficulty in establishing the time of ovulation derives from the fact that endometrial biopsies have to be carried out daily during the supposed period of ovulation. The present lack of precise knowledge as to the interval between follicular rupture and the onset of secretory changes in the endometrium adds to the difficulty of ascertaining ovulation time by this method.

## 2. Pregnanediol Excretion

Pregnanediol, an excretion product of progesterin, rises after corpus luteum formation to a peak of about 5 mg. daily and falls abruptly, shortly before the onset of the flow.

Hamblen and others [161] report their results of a 4-years' study designed to evaluate Venning's pregnanediol method as a practical and trustworthy aid in the diagnosis of progestogen levels in gynaecological and obstetric practice. Data derived from an examination of 2,193 24-hour urines of 102 patients (90 gynaecological and 12 obstetric) showed no consistent relationship between the curve of pregnanediol excretion and the predicted luteal phase of the cycle, or the degree of progestational proliferation of the endometrium and the amount of pregnanediol excreted.

When endometrial biopsies were correlated with pregnanediol determinations, it was found that of 49 patients whose bleeding occurred from progestational endometria, 21 (42.86 per cent) excreted no pregnanediol; and that of 16 patients who had bleeding from oestrogenic endometria, 10 (62.5 per cent) excreted pregnanediol in amounts of the same order as those who excreted it in association with progestational bleeding. Siegler and Bauer [162] investigated sodium pregnanediol glucuronidate excretion, using the gravimetric method of Venning with concurrent endometrial biopsies, and concluded that excretion of pregnanediol is not a positive index of ovulation or of progesterone metabolism.



### 3. Vaginal Smears

Recent interest in the vaginal smear as a diagnostic aid has led some observers to employ it as a means of ascertaining ovulation time. Smears are taken at various stages of the cycle and the cytological changes reflecting the onset of the luteal phase are taken as an indication that ovulation has occurred. The vaginal smear of the normally menstruating woman shows the following changes immediately before, during and after ovulation:

#### (a) *Pre-ovulatory Phase*

Increased proliferation of vaginal mucosa. The superficial cells show more cornification. The nuclei of the epithelial cells are smaller, stain deeper and are pyknotic; the cytoplasm has lost its granular contents, is clear and the edges are distinctly defined. Bacteria and leucocytes gradually disappear from the picture.

#### (b) *Ovulatory Phase*

Sudden decrease in oestrogen secretion, which is often reflected in the appearance of some red blood cells in the smear. In addition, there is marked desquamation of cornified cells; there are very few leucocytes and bacteria, and there are some cornified and precornified cells with folded edges.

#### (c) *Post-ovulatory Phase*

Continued desquamation of cells which are characteristically crumpled and folded together. Several types of epithelial cells are present. Some are completely cornified, the cytoplasm is wrinkled, scalloped edged and agranular, while the nuclei are small and dense. There is a progressive decrease in the number of these cells with the advance of the luteal phase. Other cells show large and oval nuclei with a wrinkled cytoplasm and scalloped edges. Their cytoplasm is granular and occasionally vacuolated. This type presumably suggests progesterone activity. There is marked influx of leucocytes and bacteria. The mucus remains abundant (see Figs. 19-21).

(Detailed methods for staining and evaluation of the smear are described on pp. 292-316.)

### 4. Basal Temperature Graphs

As early as 1904 Van der Velde discussed the variations in body temperature during phases of the menstrual cycle. During the last few years additional evidence has demonstrated the diagnostic value

of body temperature graphs. Alan Palmer [163] made observations on 35 normally menstruating women during 130 complete cycles. The temperature graphs showed a relatively constant biphasic curve with a relatively constant luteal phase independent of the length of the cycle. The women took their rectal temperature every morning on awakening. In none of the cases was there any discrepancy between the phase of the cycle as indicated by the basal temperature curve and the endometrial specimens.

Martin [164] reporting on 99 patients who had taken their vaginal or rectal temperatures during 181 cycles, found that normally menstruating women showed constant biphasic curves with a constant luteal phase. Fluctuations of temperature occurred between  $0.3^{\circ}$  and  $1^{\circ}$  F., with the following changes during the menstrual cycle:

Premenstrual phase: Fall of temperature.

Menstrual phase: Low temperature.

Oestrogenic phase: Low temperature.

Ovulatory phase: Sudden rise of temperature.

Luteal phase: Sustained elevation of temperature.

He observed that injections of oestrogen lowered, whereas injections of progesterone raised, the basal body temperature.

Ovulation is manifest in the temperature curve irrespective of whether the vaginal, rectal or oral temperature has been recorded. At the time of ovulation a steep rise occurs, with or without a preceding drop (see Figs. 43, 44). It is suggested that the lower level corresponds to the peak of oestrogen, which causes the release of the anterior pituitary gonadotrophic hormone, which in turn induces ovulation. The steep rise of temperature is presumably caused by progesterone with the onset of corpus-luteum function. It is, therefore, assumed that ovulation occurs at some point between the lower temperature level and its sudden rise. This method seems to offer a practical test for ovulation [127]; and in fact successful pregnancy often results from coitus or artificial insemination timed to synchronize with the sudden rise of temperature which follows the mid-cycle fall [125, 126, 165].

## **5. Oestrogen and Gonadotrophin in the Blood and Urine**

On the basis of the available evidence, it may be concluded that during the normal menstrual cycle, there occur two peaks of oestrogen activity which usually precede the gonadotrophin peaks. One occurs about the twelfth or thirteenth day before the flow, regardless of the



length of the cycle, and the other appears immediately before the onset of the flow. It is suggested that the rise in oestrogen stimulates the anterior pituitary to release its gonadotrophic hormones, which, in turn, cause ovulation. Ovulation, therefore, presumably occurs 24 hours after the midway peak of gonadotrophin secretion [166, 170-2].

On the basis of the increased release of gonadotrophic hormone prior to ovulation, Farris [215] developed the ovulation test.

Urine passed on rising in the morning is employed. The test animal is an immature Wistar rat between 22 and 25 days of age and weighing between 30 and 50 grams. Two c.c. of the urine are injected subcutaneously into each of two animals which are killed by illuminating-gas at the end of 2 hours. Their abdomens are opened immediately. Each ovary is inspected and its degree of redness is compared with the colours of the Munsel colour chart.

Most of the women showed a positive test for 3 or 4 successive days each month at the usually accepted ovulation time.

### **6. Uterine Reaction to Pituitrin (Knaus's Test) [167]**

This is based on the assumption that uterine reactivity to pituitrin, as determined by the intra-uterine bag method, is lost within 48 hours after follicular rupture. Chassar Moir [168] using this method, investigated the response of the human uterus to posterior lobe extract. Contrary to Knaus's observations, he found that the non-pregnant uterus responds to this extract at every phase of the menstrual cycle, although the response is most powerful immediately before, during or soon after menstruation. According to Moir, the response of an isolated muscle strip to pituitary extracts does not necessarily reflect the behaviour of the intact uterus. In particular, an isolated strip of human uterus at term has a substantially different reaction to pitocin and pitressin from that of the intact human uterus. The cause of this anomalous behaviour is unknown.

### **7. Electrometric Method [169]**

The apparatus used was a Burr-Lane-Nimms DC microvoltmeter with a General Electric photoelectric recorder as a recording galvanometer. Investigations carried out with this device showed that throughout the menstrual cycle the cervix was usually positive to the ankle from 5 to 25 MV. At the supposed time of ovulation a negative shift occurred in 9 out of 14 cycles studied, and two patients became pregnant after artificial insemination carried out on the day of the



negative shift. Further evidence, however, is required for proper evaluation of this method.

### TUBAL INSUFFLATION

Insufflation of the Fallopian tubes, first introduced by Rubin [134] is carried out for the determination of tubal patency. The apparatus comprises a cylinder of carbon dioxide whose flow is under volumetric control, and whose fluctuations and pressure are registered by a pen attached to a recording kymograph. The test should be carried out between the fourth and seventh day after cessation of menses. The technique is very simple and neither hospitalization nor anaesthesia of the patient is required.

After the introduction of a sterile speculum the cervix is painted with tincture or solution of mercurochrome in alcohol and acetone. The cervix is grasped with the tenaculum and a cannula is inserted into the cervical canal in such a manner that the rubber bulb occludes the cervix efficiently. Pressure against the cervix is maintained by attaching the tenaculum to the ratchet of the cannula.

If the tubes are patent the carbon dioxide passes into the peritoneal cavity at the pressure of about 25–40 mm. of mercury. In cases in which the tubes are partially occluded, the intra-uterine pressure first rises to a level which may reach 200 mm. of mercury and then falls sharply to 40 mm., at about which pressure the gas passes into the peritoneal cavity. Failure of carbon dioxide to pass at 200 mm. of mercury signifies occlusion of both tubes.

Auscultation of both lower abdominal quadrants with a stethoscope while the gas is passing into the tubes may give additional evidence of tubal patency by a low-pitched gurgling sound, or of tubal stenosis by a high-pitched whistling sound. These sounds must not be confounded with those due to intermittent regurgitations of gas at the cervix. Tubal patency is confirmed if, on completion of the test, the patient complains of pain usually in the right shoulder. This is referred pain due to pressure of the gas on the phrenic nerves.

Pregnancy occurs in a large number (about 25 per cent) of cases following a negative tubal insufflation test [145].

### HYSTEROSALPINGOGRAPHY

The injection of an opaque medium, for example iodized oil (at 37° C.), into the uterine cavity makes possible the radiographic ex-

amination of the uterus and tubes. The examination is carried out when carbon dioxide insufflation reveals tubal occlusion; or more frequently, without previous insufflation, for its therapeutic effect, since often pregnancy follows the injection of oil.

Usually, 10 c.c. of oil are injected slowly and carefully, pausing when the patient complains of cramping. The X-ray examination is made 2-6 hours, and again if required 24 hours, after completion of the test.

### POST-COITAL (HUHNER) TEST [135]

This test affords some indication of seminal compatibility with the vaginal and endocervical secretions.

The patient is instructed to have intercourse at about 14 days before the onset of the expected cycle, or on the day of detected ovulation, and to submit to examination within 1-2 hours later. Separate specimens are aspirated from the endocervical secretion and from the vaginal pool. The result is positive if the endocervical specimen contains spermatozoa most of which are actively motile, and if the vaginal pH is 6.2.

The pH of the normal vagina during child-bearing age is between 4.4 and 4.6; the pH of the cervical canal is between 7.3 and 8. The pH of the seminal fluid is 7.4 and 7.6. After coitus the vaginal pH changes to 6.2 for orgasm increases the cervical secretion.

### CERVICAL-SEMINAL COMPATIBILITY (MILLER-KURZROK) TEST [136]

This test is indicated in cases in which no spermatozoa are found in the endocervix, but a large number in the vagina.

A drop of semen is placed in the centre of a dry glass slide. A drop of mucus obtained from the cervix is deposited on the same slide about 3 mm. away from the semen. A cover glass is then dropped over the two liquids, bringing their margins into contact and thus setting up an immiscible phase boundary. When there is no incompatibility microscopic examination shows easy penetration of the cervical mucus by the spermatazoa which, in column formation, move forward into the plug.

In cases of incompatibility penetration does not occur and the spermatozoa appear to be actively repelled.

### CERVICAL MUCUS

The amount of cervical mucus is greatly increased at the time of ovulation [137-9].

The quantitative determination of the cervical mucus is made by aspiration into a weighed glass cannula which is inserted a few mm. into the external os.

#### Normal Values

Sixty mg. or less of mucus from the end of bleeding to about the eighth or ninth day; thereafter, a peak excretion of from 200 to 700 mg. occurs for about 4 days, followed by a sharp decline to the previous level of 60 mg. or less with no change throughout the rest of the cycle [139].

### SEMINAL EXAMINATION

The specimen is obtained by masturbation or by means of a condom. The latter is an unsatisfactory method since most condoms contain chemicals which are injurious to spermatozoa and show false necro-spermia. If psychic inhibition necessitates the use of a condom, it should be washed thoroughly with soap and water, and thereafter rinsed with tap water, the outside only being slightly powdered with talcum.

1. The seminal fluid is allowed to liquefy before the count.
2. Prepare a diluting fluid which consists of 5 per cent sodium bicarbonate and 1 per cent of formalin.
3. Draw 9.5 c.c. of diluent into a graduated pipette and transfer to a container. Then draw 0.5 c.c. of seminal fluid and add to the same container, thus making a dilution of 1 : 20.
4. After thorough homogeneity of the diluted semen is obtained, a drop is placed on the haemocytometer and a cover glass allowed to fall on the drop.
5. Three or four vertical columns consisting of 80 squares of the red blood cell field are counted and the average number calculated for 80 squares.
6. This average figure is multiplied by 1,000,000 which represents the number of spermatozoa per 1 c.c.

#### Motility

The immediate motility is estimated by the hanging drop method, using an undiluted drop of seminal fluid.



### Viability

1. Undiluted seminal fluid is placed into three small test tubes which are tightly sealed.
2. One tube is kept at room temperature, one in a refrigerator, and one in an incubator at  $37^{\circ}\text{C}$ .
3. A hanging drop preparation of each tube is examined for motility every 4–8 hours until motility ceases.

### STAINING OF SPERMATOZOA

#### Hamblen's Method

Various methods are used for the staining of spermatozoa. Hamblen [140] describes the following procedure which takes only 25 minutes [141].

After the smear is fixed with heat, the staining technique proceeds throughout in Coplin jars as follows:

1. Let stand in 95 per cent alcohol 5–10 minutes.
2. Wash out excess alcohol in tap water.
3. Stain for 1 minute in Harris's haematoxylin (Harris's haematoxylin, 1 part; 8 per cent alcohol, 3 parts). (In making the original stock solution of Harris's haematoxylin 4 c.c. of glacial acetic acid are added to 100 c.c. of stain).
4. Rinse out excess haematoxylin in distilled water.
5. Destain 5–7 seconds in acid alcohol (concentrated hydrochloric acid, 1 c.c.; 95 c.c. of 70 per cent alcohol).
6. Rinse thoroughly in distilled water.
7. Transfer to weak alkaline water (ammonium hydroxide, specific gravity 0.90, 2 drops; tap water, 60 c.c.) for 5–10 seconds.
8. Rinse in distilled water.
9. Counter-stain in eosin (eosin "Y", 1 gram; 25 per cent alcohol, 95 c.c.) for 3 minutes.
10. Rinse quickly in distilled water.
11. Counter-stain in 1 per cent aqueous solution of fast green for 15–20 seconds.
12. Rinse in 95 per cent alcohol; leave stain streaking on slide.
13. Rinse in absolute alcohol until, upon gross inspection, the green dye has been washed out. This requires a few seconds.
14. Clear for 3 minutes in xylol.
15. Mount in balsam.

The spermatozoa are differentiated by this stain as follows:

Acrosome—lavender; nucleus—pink; body and tail—green.

### Other Methods

Greenberg and others [142] recently reported another modification of spermatozoa staining.

#### FORMULA AND PREPARATION

methyl green, dye content 60 per cent . . . . .	1.0 gram
pyronine, bluish, certified . . . . .	0.2 gram
methyl alcohol, absolute . . . . .	10.0 c.c.
phenol, 2 per cent aqueous solution . . . . .	100.0 c.c.
glycerol, cp. . . . .	20.0 c.c.

Dissolve the dyes in the alcohol and phenol by intermittent shaking about 2 hours each day for 2 days, using a mechanical shaker. Filter and add the glycerine. Refilter into dropping bottle as the supply is needed.

An alternate formula is as follows:

methyl green, dye content 60 per cent . . . . .	1.0 gram
pyronine, bluish, certified . . . . .	0.25 gram
alcohol, 95 per cent . . . . .	5.0 c.c.
glycerol, cp. . . . .	20.0 c.c.
phenol, 2 per cent aqueous solution . . . . .	100.0 c.c.

Heat solvents to about 60° C. Rub dyes together and add to solvents. Agitate until dyes are dissolved. The solution should be allowed to stand for an hour or more before filtering.

#### *Procedure:*

1. Prepare a smear on a clean dry slide. Dry in air.
2. Fix in a solution of 1 part 95 per cent alcohol, 1 part ether, and dry in air; or fix by heat.
3. Flood the slide with the stain. If desired, heat the slide until steam rises from the stain. This latter step is not necessary but results in better staining of some cellular elements. Let stand 5 minutes.
4. Wash with tap water.
5. Dry and examine with oil immersion lens.

### Microscopic Appearance of Spermatozoa

The sperm-head is coloured blue-green, the acrosome lighter than the posterior part. The neck and tail are pink, less distinct than the head but clearly visible. It is suggested that there may be a tendency for abnormal forms to stain more darkly than the normal.

### Normal Seminal Values

Volume: 3–4 c.c.

Motility: 80–95 per cent.

Viability: 12–24 hours' active motility.

Count: 60,000,000–100,000,000 per 1 c.c.

Morphology: less than 20 per cent abnormal forms.

pH: 7.5–7.8.

Hamblen [143] reports the following average findings in sub-fertile husbands whose wives later became pregnant:

Volume: 1–8 c.c.

Motility: 8–95 per cent.

Count: 2,000,000–249,000,000 per 1 c.c.

### EPIDIDYMAL AND TESTICULAR PUNCTURE

This is useful in the differential diagnosis between bilateral duct occlusion and complete azoospermia. After painting the overlying scrotum with antiseptic solution and anaesthetizing by injection of a few drops of procaine hydrochloride, a hypodermic needle is inserted into the epididymis or testis and the seminal fluid aspirated by the attached syringe.

### TESTICULAR BIOPSY

By this method it is possible to make an histological evaluation of spermatogenesis and the degree of tubular atrophy.

Under strict asepsis and procaine anaesthesia, an incision about half an inch in length is made in the tightly held skin of the scrotum to expose the testis. A minute incision is then made in the tunica albuginea and, by slight pressure on the testis, a small amount of yellow frothy-looking seminal tissue is forced out. A small shaving is taken from this tissue and the specimen is then placed in fixing solution (10 per cent formaldehyde or Bouin's solution) for microscopic study. The testis is allowed to fall back and a single suture applied to the scrotal incision. A light cotton dressing with collodion is used and an athletic support applied.

Various grades of impairment in the seminal epithelium may be found on histological examination [144].

1. Spermatogenesis may be arrested at the spermatid stage.
2. Spermatogenesis may be arrested at an earlier stage.



3. The seminiferous tubules may be practically devoid of germinal epithelium and their lumen filled with hyaline. There may be varying grades of fibrosis.

## HYALURONIDASE DETERMINATION IN SEMEN AND TISSUE EXTRACTS BY THE TURBIDIMETRIC METHOD [216]

### *Reagents*

Hyaluronic acid solution. Dissolve 20.0 mg. hyaluronic acid in 50 c.c. 0.1M acetate buffer (pH 6.0) and warm the solution for several hours at approximately 37° C. to effect complete solution. Centrifuge or filter the solution to remove lint or other particles. The solution is stable for several weeks if stored at low temperature (10° C.).

Hyaluronic acid may be obtained from several sources: from vitreous humour of cattle eyes, synovial fluid, or human umbilical cords. Vitreous humour is most satisfactory in this work because of availability and ease of preparation.

0.5M acetic acid.

0.5M sodium acetate solution.

0.1M acetate buffer (pH 6.0). Mix one part 0.5M acetic acid with 20 parts 0.5M sodium acetate solution and dilute the solution 1 : 5.

0.5M acetate buffer (pH 4.2). Mix 28 parts 0.5M acetic acid with 0.5M sodium acetate solution.

Acidified blood serum. Add 5 c.c. of fresh horse, rabbit, or sheep serum to 45 c.c. 0.5M acetate buffer (pH 4.2) and adjust the pH of the solution to 4.2 with the glass electrode. The solution should stand for at least 24 hours before use.

It is best that only fresh serum (no more than 2-3 weeks old) be used because the serum, acidified or unacidified, undergoes a change which produces lowered, and therefore frequently unusable turbidity values.

### *Apparatus*

Colorimeter. For determining turbidity, a Klett-Summerson Photoelectric Colorimeter with red filter No. 66 is used.

Colorimeter tubes. It is imperative to use carefully matched tubes. The so-called "matched" tubes obtainable from supply houses may vary as much as 10 scale units. To circumvent this situation, one tube is selected as the reference tube and all others are compared to it. The tubes are classified into the following three groups: those exhibiting differences of 0-3, 4-6, and 7-8 scale-units. Determinations are made

in tubes of one group, otherwise appropriate correction factors have to be introduced.

Oven. The enzymatic reactions are carried out in an oven at a temperature of 37° C.

### *Turbidimetric Technique*

To assay a given enzyme preparation, the enzyme solution of selected concentration and the hyaluronic acid solution are brought to 37° C. In a colorimeter tube 0.5 c.c. of the enzyme solution and 0.5 c.c. of substrate (containing 0.2 mg. of hyaluronic acid) are mixed well and incubated at 37° C. for 3.0 minutes. The tubes are then chilled in an ice-bath for 5 minutes and 30 c.c. pH 4.2 acetate buffer followed by 1 c.c. acidified blood serum are added. The contents of the tube are mixed by inverting the tube three times. The turbidity is permitted to develop at room temperature for 30 minutes and is then measured in the colorimeter. The turbidity value is the number of scale-divisions read on the colorimeter scale.

In order to equate the scale reading obtained to a known turbidity value, it is required that two standards be prepared. These are designated as the 0.2 c.c. standard and the 0.1 c.c. standard. They are prepared during the running of the determination and for any one day's work it is only necessary that the values of these standards be determined once. The 0.2 c.c. standard is prepared by placing 0.5 c.c. substrate solution in a colorimeter tube, and adding, in order: 0.5 c.c. pH 6.0 acetate buffer, 3.0 c.c. pH 4.2 acetate buffer, and 1 c.c. acidified blood serum. The 0.1 c.c. standard is prepared in a similar manner excepting that 0.25 c.c. substrate solution and 0.75 pH 6.0 acetate buffer is used instead. The turbidity value is read at the end of 30 minutes. The scale readings correspond to the turbidities of 0.2 and 0.1 c.c. hyaluronic acid; and that concentration of enzyme which reduces the turbidity produced by 0.2 c.c. hyaluronic acid to the turbidity produced by 0.1 c.c. hyaluronic acid is said to contain one turbidity-reducing unit—TRU.

With the preparation of hyaluronidase used, it was found that 1 rat-ova unit and the TRU under these conditions were the same (0.033 mg.).

### **Rat-ova Test [217]**

The following method for assaying hyaluronidase activity by the rat-ova test (dispersal of the follicle cells) is described.

The eggs removed from each oviduct are placed in the separate concavities of a duplex depression slide, washed in Ringer's solution,

and covered with 0.1 c.c. of this solution. The slide is kept in a petri dish together with a piece of moistened filter paper when not under examination. At least 3 eggs are placed in each chamber. If fewer eggs are obtained from any one oviduct they are redistributed. The eggs usually adhere in a stringy mass, but by the use of iridectomy scissors a given number can be cut out and transferred with a small glass pipette without fear of removing an appreciable number of follicle cells.

A series of enzyme dilutions in Ringer's is prepared and 0.1 c.c. added to each group of eggs so that the total volume of fluid is 0.2 c.c. The enzyme is stirred in with a needle and if the eggs are suspended in the surface film, they are gently pushed to the bottom of the depression slide. The slides are examined at intervals of  $\frac{1}{2}$ ,  $\frac{3}{4}$ , 1,  $1\frac{1}{2}$  and 2 hours, and longer if necessary. The end-point is established when 2 of 3 eggs, or 50 per cent of a greater number (not exceeding 8), have lost their follicle cells to a degree varying from complete denudation to where not more than a single layer of follicle cells remains. A unit of hyaluronidase by the rat-ova test is defined as the amount of hyaluronidase in 0.2 c.c. of Ringer's solution which gives an end-point, as defined above, within  $\frac{3}{4}$ -1 hour.

McClellan and Rowlands [218] demonstrated in mammalian testis the presence of hyaluronidase and its capacity to liquefy the highly viscous gel which cements the cumulus cells and corona radiata around the unfertilized tubal egg of the rat. Rowlands [181] was able markedly to decrease the number of sperms necessary to fertilize rabbits successfully by artificial insemination, when an extract of rabbit sperms containing hyaluronidase was added to the diluted sperm suspension. In man there is usually a parallelism between the number of sperms and the hyaluronidase content of the semen. Semen which does not contain sperms shows no hyaluronidase activity [83]. This explains the rapid decrease of fertility in males whose semen contains less than 60 million sperms per c.c.

## BIBLIOGRAPHY

1. FLUHMAN, C. J. *J.A.M.A.*, **92**, 1744, 1929; *Am. J. Obst. & Gynec.*, **20**, 1, 1930.
2. FRANK, R. T., and SALMON, U. J. *Proc. Soc. Exper. Biol. & Med.*, **34**, 363, 1936.
3. FRANK, R. T., and BERMAN, R. L. *Endocrinol.*, **25**, 996, 1939.
4. VARNEY, R., and KOCH, F. C. *Endocrinol.*, **30**, 399, 1942.



5. KLINEFELTER, H. F., JR., ALBRIGHT, F., and GRISWOLD, G. C. *J. Clin. Endocrinol.*, **3**, 529, 1943.
6. ASCHHEIM, S., and ZONDEK, B. *Klin. Wchnschr.*, **7**, 1401, 1928.
7. FRIEDMAN, M. H., and LAPHAM, M. E. *Am. J. Obst. & Gynec.*, **21**, 405, 1931.
8. SIMPSON, S. L. *Brit. Postgrad. Med. J.*, **20**, 163, 1944.
9. HOFFMAN, J. "Female Endocrinology", W. B. Saunders Co., Philadelphia, 1944.
10. SALMON, U. J., GEIST, S. H., SALMON, A. A., and FRANK, T. L. *J. Clin. Endocrinol.*, **2**, 169, 1942.
11. KUPPERMAN, H. S., GREENBLATT, R. B., and NOBACK, C. R. *J. Clin. Endocrinol.*, **3**, 548, 1943.  
KUPPERMAN, H. S., and GREENBLATT, R. B. *South. Med. J.*, **39**, 158, 1946.
12. KAMINESTER, S. A. *Am. J. Obst. & Gynec.*, **47**, 265, 1944.
13. FARRIS, E. J. *Am. J. Obst. & Gynec.*, **48**, 200, 1944.
14. HOGBEN, L. *Proc. Roy. Soc. Med. S. Africa*, **5**, 19, 1930.
15. ZONDEK, B., SULMAN, F., and BLACK, R. *J.A.M.A.*, **128**, 939, 1945.
16. LANDGREBE, F. W., and SAMSON, L. J. *Obst. & Gynec. Brit. Emp.*, **51**, 133, 1944.
17. WEISSMAN, A. S., SNYDER, A. F., and COATES, C. W. *Am. J. Obst. & Gynec.*, **43**, 135, 1942.
18. ZWARENSTEIN, H., and DUNCAN, D. G. *Clinical Proc.*, **3**, 186, 1944.
19. SCOTT, L. D. *Brit. J. Exper. Path.*, **21**, 320, 1940.
20. GEOGHEGAN, F., and MCGRATH, J. *Irish J. Med. Sc.*, 6th series, No. 225, 485, 1944.
21. WEISSMAN, A. G., and COATES, C. W. *J. Clin. Endocrinol.*, **4**, 35, 1944.
22. GUTERMAN, H. S. *J. Clin. Endocrinol.*, **4**, 262, 1944.
23. HAIG, CARAPENTYAN. *J.A.M.A.*, **122**, 81, 1943.
24. WINKLESTEIN, L. B. *Am. J. Obst. & Gynec.*, **44**, 231, 1942.
25. GROSSMAN, L. L. *West. J. Surg.*, **52**, 443, 1944.
26. PAGE, E. W. *West. J. Surg.*, **51**, 482, 1943.
27. GOODFRIEND, J. R., and DANIEL, M. *Am. J. Obst. & Gynec.*, **45**, 140, 1943.
28. ALLEN, E., and DOISY, E. A. *J.A.M.A.*, **81**, 819, 1923.
29. ASTWOOD, E. B. *Endocrinol.*, **23**, 25, 1938.
30. FRANK, R. T., and GOLDBERGER, M. A. *Proc. Soc. Exper. Biol. & Med.*, **32**, 1663, 1935.
31. FLUHMAN, C. F. *Endocrinol.*, **18**, 705, 1934.
32. SMITH, G. V., and SMITH, O. W. *Am. J. Physiol.*, **112**, 340, 1935.
33. MAZER, C., and HOFFMAN, J. *Am. J. Obst. & Gynec.*, **17**, 186, 1929.
34. KURZROK, R., and RATNER, A. *Am. J. Obst. & Gynec.*, **28**, 689, 1932.
35. CHERRY, T. H., and BERNSTEIN, M. J. *Proc. Soc. Exper. Biol. & Med.*, **40**, 688, 1939.
36. WERNER, S. C. *J. Clin. Invest.*, **20**, 21, 1941.
37. BACHMAN, C., and PETTIT, D. S. *J. Biol. & Chem.*, **138**, 689, 1941.

38. D'AMOUR, F. E. *Am. J. Obst. & Gynec.*, **40**, 958, 1940.
39. SMITH, O. W., SMITH, G. V., and SCHILLER, S. *Endocrinol.*, **25**, 509, 1939.
40. SMITH, O. W., SMITH, G. V., and SCHILLER, S. *J. Clin. Endocrinol.*, **1**, 461, 1941.
41. VENNING, E. H. *J. Biol. Chem.*, **119**, 473, 1937; **126**, 595, 1938.
42. BUCHNER, N. L. R., and GESCHICHTER, C. F. *Endocrinol.*, **27**, 727, 1940.
43. ASTWOOD, E. B., and JONES, G. E. *J. Biol. Chem.*, **137**, 397, 1941.
44. HAMBLIN, E. C., CUYLER, W. K., and BAPTIST, M. *Am. J. Obst. & Gynec.*, **44**, 442, 1941.
45. SIEGLER, S. L., and BAUER, D. *Am. J. Obst. & Gynec.*, **45**, 277, 1943.
46. HAMBLIN, E. C., CUYLER, W. K., and BAPTIST, M. *Am. J. Obst. & Gynec.*, **44**, 442, 1941.
47. CALLOW, R. K. *Lancet*, **2**, 565, 1936.
48. FRANK, R. T., KLEMPNER, E., HOLLANDER, F., and KRISS, B. *Endocrinol.*, **31**, 63, 1942.
49. ZIMMERMANN, W. *Ztschr. f. physiol. Chem.*, **233**, 257, 1935.
50. CHOU, C. Y., and WU, H. *Chinese J. Physiol.*, **11**, 429, 1937.
51. TALBOT, N. B., BUTLER, A. M., MACLACHLAN, E. A., and STROUD, S. W. *J. of Endocrinol.*, **1**, 76, 1939.
52. FRIEDGOOD, H. B., TAYLOR, E. H., and WRIGHT, M. L. *J. Clin. Endocrinol.*, **3**, 638, 1943.
53. WOLFE, J. K., RIESER, L. F., and FRIEDGOOD, H. B. *J. Am. Chem. Soc.*, **63**, 582, 1941.
54. VENNING, E. H., HOFFMANN, M. M., and BROWNE, J. S. L. *Federation Proc.*, **1**, 139, 1942; *J. Biol. Chem.*, **146**, 369, 1942.
55. ROBBIE, W. A., and GIBSON, R. B. *J. Clin. Endocrinol.*, **3**, 200, 1943.
56. CALLOW, N. H. and R. K. and GUINNESS, C. W. *Biochem. J.*, **32**, 1312, 1938.
57. BAUMAN, E. J., and METZGER, N. *Endocrinol.*, **27**, 664, 1940.
58. HOLTORFF, A. E., and KOCH, F. C. *J. Biol. Chem.*, **135**, 377, 1940.
59. TALBOT, N. B., BUTLER, A. M., and MACLACHLAN, E. *J. Biol. Chem.*, **132**, 595, 1940.
60. WOOSTER, H. *J. Clin. Endocrinol.*, **3**, 483, 1943.
61. HERSCHBERG, E. B., and WOLFE, J. K. *J. Biol. Chem.*, **133**, 667, 1940.
62. CALLOW, N. H. and R. K., EMMENS, C. W., and STROUD, S. W. *J. Endocrinol.*, **1**, 76, 1939.
63. TALBOT, N. B., BERMAN, R. A., and MACLACHLAN, E. A. *J. Biol. Chem.*, **143**, 211, 1942.
64. TALBOT, N. B., BUTLER, A. M., BERMAN, R. A., RODRIGUEZ, P. M., and MACLACHLAN, E. A. *Am. J. Dis. Child.*, **65**, 64, 1943.
65. WERNER, S. C. *J. Clin. Endocrinol.*, **1**, 951, 1941.
66. FRASER, R. W., FORBES, A. P., ALBRIGHT, F., SULKOWITCH, H., and REIFENSTEIN, E. C., JR. *J. Clin. Endocrinol.*, **1**, 234, 1941.
67. CUTLER, H. H., POWER, M. H., and WILDER, R. M. *J.A.M.A.*, **111**, 117, 1938.
68. MENCHER, W. H. *J.A.M.A.*, **109**, 1338, 1937.

69. PAPANICOLAOU, G. N., and TRAUT, H. F. "Diagnosis of Uterine Cancer by the Vaginal Smear", The Commonwealth Fund, 1943.
70. SHORR, E. *Science*, **91**, 579, 1940.
71. *Ibid.*, **94**, 545, 1941.
72. MACK, H. C. *Harper Hosp. Bull.*, **1**, 54, 1942; *J. Clin. Endocrinol.*, **2**, 361, 1942; *ibid.*, **3**, 169, 1943.
73. MACK, H. C., and ALE, T. *J. Clin. Endocrinol.*, **2**, 361, 1942.
74. RUBINSTEIN, B. B. *Endocrinol.*, **27**, 843, 1940.
75. PAPANICOLAOU, G. N. *Proc. Soc. Exper. Biol. & Med.*, **22**, 436, 1925.
76. SMITH, B. J., and BRUNNER, E. K. *Am. J. Anat.*, **54**, 27, 1934.
77. MACK, H. C. *Am. J. Obst. & Gynec.*, **45**, 402, 1943.
78. HALL, G. J. *J. Clin. Endocrinol.*, **5**, 34, 1945.
79. SCHUMAN, W. *Am. J. Obst. & Gynec.*, **47**, 808, 1944.
80. PAPANICOLAOU, G. N. *Science*, **95**, 438, 1942.
81. SHORR, E. *J. Mount Sinai Hospital*, **12**, 667, 1945.
82. JONES, C. A., NEUSTALDER, T., and MACKENZIE, L. L. *Am. J. Obst. & Gynec.*, **49**, 159, 1945.
83. EICHENBERGER, E. *Gynaecologia, Basel*, **121**, 288, 1946.
84. KATZMAN, P. A., and DOISY, E. A. *J. Biol. Chem.*, **106**, 125, 1934.
85. CUTLER, M., and OWEN, S. E. *Am. J. Cancer*, **24**, 318, 1935.
86. GLASS, S. J., and MCKENNON, D. J. *West. J. Surg.*, **45**, 467, 1937.
87. FRIEDMAN, M. H., and WEINSTEIN, J. L. *Endocrinol.*, **21**, 489, 1937.
88. FRANK, R. T., and SALMON, U. J. *Proc. Soc. Exper. Biol. & Med.*, **32**, 1237, 1935.
89. FRANK, R. T. "Glandular Physiology and Therapy", Am. Med. Assn., Chicago, Ill., 1935.
90. DRIPS, D. J., and OSTERBERG, A. E. *Endocrinol.*, **23**, 703, 1938.
91. D'AMOUR, F. E. *J. Clin. Endocrinol.*, **3**, 41, 1943.
92. HOFFMAN, J. "Female Endocrinology", W. B. Saunders Co., Philadelphia, 1944.
93. EVANS, H. T., KOHLS, C. L., and WONDER, D. H. *J.A.M.A.*, **108**, 287, 1937.
94. RAKOFF, A. E. *Pennsylvania M.J.*, **43**, 669, 1940.
95. BROWNE, G. S. L., and VENNING, E. H. *Lancet*, **2**, 1507, 1936.
96. FLUHMAN, C. F. *Am. J. Obst. & Gynec.*, **20**, 1, 1930.
97. ZONDEK, B. *Klin. Wchnschr.*, **9**, 393, 1930.
98. VARNEY, R. F., KENYON, A. T., and KOCH, F. C. *J. Clin. Endocrinol.*, **2**, 137, 1942.
99. PALMER, A. *Proc. Soc. Exper. Biol. & Med.*, **37**, 273, 1937.
100. ZONDEK, B., and EULER, H. *Skandinav. Anat. J. Physiol.*, **67**, 259, 1934.
101. FRANK, R. T., and GOLDBERGER, M. A. *Am. J. Obst. & Gynec.*, **43**, 865, 1942.
102. COHEN, S. L., MARRIANS, J. F., and WATSON, M. *Lancet*, **1**, 674, 1935.
103. RAKOFF, A. E., PASCHKIS, K. E., and CANTAROW, A. *Am. J. Obst. & Gynec.*, **46**, 856, 1943.
104. BROWNE, J. S. L., and VENNING, E. H. *Am. J. Physiol.*, **116**, 18, 1936.



105. WATSON, B. A., YOLTON, N., and RAULS, L. *J. Lab. & Clin. Med.*, **28**, 732, 1943.
106. ENG, H. *Klin. Wchnschr.*, **15**, 349, 1936.
107. FRANK, R. T., GOLDBERGER, M. A., and SPIELMAN, F. *J.A.M.A.*, **103**, 393, 1934.
108. LAROCHE, J., SIMONNET, H., and HUEB, J. A. *Compt. rend. Soc. de Biol.*, **113**, 286, 1933.
109. KOCH, F. C. *Ann. Int. Med.*, **11**, 297, 1937.
110. BROWNE, J. S. L., and VENNING, E. H. *Endocrinol.*, **21**, 7111, 1937; *J. Clin. Invest.*, **16**, 678, 1937.
111. BROWNE, J. S. L., HENRY, J. S., and VENNING, E. H. *Am. J. Obst. & Gynec.*, **38**, 927, 1939.
112. GALLAGHER, T. F., PETERSON, D. H., DORFMAN, R. T., KENYON, A. T., and KOCH, F. C. *J. Clin. Invest.*, **16**, 695, 1937.
113. DINGEMANSE, E., BORCHARDT, H., and LAQUEUR, E. *Biochem. J.*, **31**, 500, 1937.
114. KOCH, F. C. *Biol. Symposia*, **9**, 41, 1942.
115. ZONDEK, B., and ASCHHEIM, S. *Klin. Wchnschr.*, **7**, 485, 1928.
116. OESTING, R. B., and WEBSTER, B. *Endocrinol.*, **22**, 307, 1938.
117. FRIEDGOOD, H. B., and WHIDDEN, H. K. *Endocrinol.*, **25**, 919, 1939.
118. CUTLER, W. K., and BAPTIST, M. *J. Lab. & Clin. Med.*, **26**, 881, 1941.
119. PINCUS, J. *Endocrinol.*, **32**, 176, 1943.
120. HOFFMAN, J. "Female Endocrinology", W. B. Saunders Co., Philadelphia, 1944.
121. PALMER, ALAN. *Surg. Gynec. & Obst.*, **75**, 768, 1942.
122. PURVIS, L. M. *Am. J. Obst. & Gynec.*, **46**, 53, 1943.
123. WILLIAMS, W. W. *Am. J. Obst. & Gynec.*, **46**, 662, 1943.
124. LYON, R. *Am. J. Obst. & Gynec.*, **76**, 729, 1943.
125. TOMPKINS, P. *J. Am. Med. Assn.*, **124**, 698, 1944.
126. TOMPKINS, P. *J. Obst. & Gynec. Brit. Emp.*, **52**, 241, 1945.
127. NIEBURGS, H. E. *J. Obst. & Gynec. Brit. Emp.*, **52**, 435, 1945.
128. DRAPER, G., DUPERTUIS, C. W., and CAUGHEY, J. L., JR. "Human Constitution in Clinical Medicine", Paul. B. Hoeber, Inc., New York and London, 1944.
129. WERNER, A. A. "Endocrinology", H. Kimpton, London, 1942.
130. VON ARVAY, A., and MEYER, H. *Zentralbl. f. Gynäk.*, **56**, 194, 1932.
131. DUNCAN, G. G. "Diseases of Metabolism", W. B. Saunders Co., Philadelphia, 1942.
132. MEANS, J. H., and BURGESS, H. W. *Arch. Int. Med.*, **30**, 507, 1922.
133. LIPTON, R. F., and ABEL, M. S. *Am. J. Sci.*, **208**, 736, 1944.
134. RUBIN, S. C. *J.A.M.A.*, **75**, 661, 1920.
135. HÜHNER, M. "Sterility in the Male and Female", Rebman & Co., New York, 1913.
136. KURZROK, R. "The Endocrines in Obstetrics and Gynaecology", Williams & Wilkins Co., Baltimore, 1937.
137. SEGUY, J., and VIMEUX, J. *Gynec. & Obst.*, **27**, 346, 1937.

138. SEGUY, J., and SIMONNET, H. *Gynec. & Obst.*, **28**, 657, 1943.
139. VIERGIVER, E., and POMMERENKE, W. *Am. J. Obst. & Gynec.*, **48**, 321, 1944.
140. HAMBLÉN, E. C. "Endocrinology of Women," Chas. C. Thomas, Springfield, Illinois, 1945.
141. CUYLER, K., and BAPTIST, M. *J. Clin. Endocrinol.*, **2**, 571, 1942.
142. GREENBERG, B. E., BERMANS, S., GARGILL, S. L., and GRIFFIN, R. C. *J. Clin. Endocrinol.*, **3**, 179, 1943.
143. HAMBLÉN, E. C. *South. Med. J.*, **34**, 1229, 1941.
144. HOTCHKISS, R. S. *Bull. N.Y. Acad. Med.*, **18**, 600, 1942.
145. SHARMAN, A. *J. Obst. & Gynec. Brit. Emp.*, **51**, 85, 1944.
146. HELLER, C. G., and HELLER, E. J. *Endocrinol.*, **24**, 319, 1939.
147. CRUICKSHANK, R., and SHERMAN, A. J. *J. Obst. & Gynec. Brit. Emp.*, **41**, 208, 1934.
148. HISAW, F. L., and GREEP, R. O. *Proc. Soc. Exper. Biol. & Med.*, **35**, 29, 1936.
149. HISAW, F. L., GREEP, R. O., and FEVOLD, H. L. *Am. J. Anat.*, **61**, 483, 1937.
150. OGINO, L. *Zentralbl. f. Gynäk.*, **56**, 721, 1932.
151. OGINO, L. *Am. J. Obst. & Gynec.*, **37**, 940, 1939.
152. GROSSE, O. *Am. J. Obst. & Gynec.*, **13**, 356, 1927.
153. DICKINSON, R. L. *Am. J. Obst. & Gynec.*, **14**, 718, 1927.
154. MAZER, C., and ISRAEL, S. L. "Menstrual Disorders and Sterility", p. 70, Heinemann, London, 1941.
155. KURZROK, R. "Endocrines in Obstetrics and Gynecology", p. 210, Williams & Wilkins Co., Baltimore, 1937.
156. BIRNBERG, C. H. *Endocrinol.*, **21**, 294, 1937.
157. GREENE-ARMITAGE. Personal Communication.
158. ZONDEK, H. "Genital Functions and the Hormonal Regulation", p. 229, Williams & Wilkins Co., Baltimore, 1941.
159. WESTMAN, A. *Arch. Gynec.*, **156**, 550, 1934.
160. ZONDEK, B. *J. Physiol.*, **81**, 4, 1943.
161. HAMBLÉN, E. C., GAYLER, W. K., and BAPTIST, M. *Am. J. Obst. & Gynec.*, **44**, 442, 1941.
162. SIEGLER, S. L., and BAUER, D. *Am. J. Obst. & Gynec.*, **45**, 277, 1943.
163. PALMER, ALAN. *Surg. Gynec. & Obst.*, **75**, 768, 1942.
164. PURVIS, L. MARTIN. *Am. J. Obst. & Gynec.*, **46**, 53, 1943.
165. BARTON, M., WALKER, K., and WIESNER, B. P. *Brit. Med. J.*, 4384, 40, 1945.
166. SMITH, G. V. S., and SMITH, W. O. *New England Med. J.*, **215**, 908, 1936.
167. KNAUS, H. *Zentralbl. f. Gynäk.*, **59**, 2642, 1935.
168. CHASSAR, MOIR. *J. Obst. & Gynec. Brit. Emp.*, **3**, 181, 1944.
169. LANGMAN, L., and BURR, H. S. *Am. J. Obst. & Gynec.*, **44**, 223, 1942.
170. KURZROK, R. *Am. J. Obst. & Gynec.*, **28**, 319, 1934.
171. FRANK, R. T. *J.A.M.A.*, **103**, 393, 1934.
172. D'AMOUR, F. E. *Am. J. Obst. & Gynec.*, **40**, 958, 1940.

173. JONES, G. E. S., DELFS, E., and STRAN, H. M. *Bull. Johns Hopkins Hosp.*, **75**, 359, 1944.
174. HARTMAN, C. G., and LITRELL, J. *Science*, **102**, 178, 1945.
175. MARKEE, J. E., and BERG, B. *Stanford Med. Bull.*, **2**, 55, 1944.
176. ESPINASSE, P. G. *Nature*, **144**, 1013, 1939.
177. PAPANICOLAOU, G. N., and SHORR, E. *Am. J. Obst. & Gynec.*, **38**, 392, 1939.
178. SALMON, N. G., WALTER, R. J., and GEIST, S. H. *Proc. Soc. Exper. Biol. & Med.*, **39**, 467, 1939.
179. MEIGS, J. V., GRAHAM, R. M., FREMONT-SMITH, M., KAPNICK, J., and RAWSON, R. W. *Surg. Gyn. & Obst.*, **77**, 449, 1943.
180. MEIGS, G. V., GRAHAM, R. M., FREMONT-SMITH, M., JANZEN, L. T., and NELSON, C. B. *Surg. Gyn. & Obst.*, **81**, 337, 1945.
181. ROWLANDS, I. W. *Nature*, **154**, 332, 1944.
182. GORBMAN, A. *Endocrinol.*, **37**, 177, 1945.
183. JUNGCK, E. C., MADDOCK, W. O., and HELLER, C. G. *J. Clin. Endocrinol.*, **7**, 1, 1947.
184. ROBBINS, S. L., PARKES, F., JR., and BIANCO, P. D. *Endocrinol.*, **40**, 227, 1947.
185. ZONDEK, B., and SULMAN, F. *J. Clin. Endocrinol.*, **7**, 79, 1947.
186. BURDICK, H. O. *Am. J. Physiol.*, **145**, 387, 1946.
187. BUNDE, C. A. *Am. J. Obst. & Gynec.*, **53**, 317, 1947.
188. FOOTE, E. C., and SEEGAR-JONES, G. E. *Am. J. Obst. & Gynec.*, **51**, 672, 1946.
189. GUTERMAN, H. S. *J. Clin. Endocrinol.*, **5**, 407, 1945.
190. MCCORMACK, G. *Am. J. Obst. & Gynec.*, **51**, 722, 1946.
191. MORROW, A. G., and BENUA, R. S. *Am. J. Obst. & Gynec.*, **51**, 685, 1946.
192. GUTERMAN, H. S. *Am. J. Obst. & Gynec.*, **52**, 174, 1946.
193. HARTMAN, C. G., LITRELL, I. L., and TORN, I. *Endocrinol.*, **39**, 120, 1946.
194. LLOYD, C. W., ROGERS, W. F., JR., and WILLIAMS, R. H. *Endocrinol.*, **39**, 256, 1946.
195. FINKELSTEIN, M., HESTRIN, S., and KOCH, W. *Proc. Soc. Exper. Biol. & Med.*, **64**, 64, 1947.
196. BATES, R. W., and COHEN, H. *Fed. Proc. Biochem. Soc.*, Chicago, **6**, 236, 1947.
197. COHEN, H., and BATES, R. W. Twenty-Ninth Ann. Meet. of Assn. for Study of Internal Secretions, 1947.
198. WARREN, F. L. *Cancer Research*, **5**, 49, 1945.
199. CALLOW, N. H. and R. K., and EMMENS, C. W. *Biochem. J.*, **32**, 1312, 1938.
200. DREKTER, I., PEARSON, S., BARTZCAK, E., and MCGAVACK, T. H. Twenty-Ninth Ann. Meet. of Assn. for Study of Internal Secretions, 1947.
201. VENNING, E. H., KAZMIN, V. E., and BELL, I. C. *Endocrinol.*, **38**, 79, 1946.
202. DORFMAN, R. I., ROSS, E., and SHIPLEY, R. A. *Endocrinol.*, **38**, 178, 1946.



203. EGGLESTON, N. M., JOHNSTON, B. I., and DOBRINER, K. *Endocrinol.*, **38**, 197, 1946.
204. DORFMAN, R. I., SHIPLEY, R. A., SCHILLER, S., and HORWITT, N. *Endocrinol.*, **38**, 165, 1946.
205. ROBINSON, F. I., POWER, M. H., and KEPLER, E. I. *Proc. Staff Meet., Mayo Clin.*, **16**, 577, 1941.
206. LEVY, M. S., POWER, M. H., and KEPLER, E. I. *J. Clin. Endocrinol.*, **6**, 607, 1946.
207. NELSON, W. O., and GALLAGHER, T. F. *Science*, **84**, 230, 1936.
208. KORENCHEVSKY, V., and HALL, K. *J. Path. & Bact.*, **45**, 681, 1937.
209. MACDONALD, A. M., and ROBSON, I. M. *J. Path. & Bact.*, **48**, 95, 1939.
210. NIEBURGS, H. E. *J. Obst. & Gynec. Brit. Emp.*, **54**, 653, 1947.
211. AYRE, I. E., and DAKIN, E. *The Canad. Med. Assn. J.*, **54**, 489, 1946.
212. INES, L. C. de ALLENDE, SHORR, E., and HARTMAN, C. G. *Contributions to Embryology*, **31**, 1, 1943.
213. AYRE, I. E. *Am. J. Obst. & Gynec.*, **51**, 743, 1946.
214. AYRE, I. E. *South. Med. J.*, **39**, 847, 1946.
215. FARRIS, E. J. *Am. J. Obst. & Gynec.*, **52**, 14, 1946.
216. LEONARD, S. L., PERLMAN, P. L., and KURZROK, R. *Endocrinol.*, **39**, 261, 1946.
217. LEONARD, S. L., and KURZROK, R. *Endocrinol.*, **39**, 85, 1946.
218. MCCLEAN, D., and ROWLANDS, I. W. *Nature*, **150**, 627, 1942.
219. NIEBURGS, H. E., and GREENBLATT, R. B. *South Med. J.*, **41**, 972, 1948.
220. NIEBURGS, H. E., and GREENBLATT, R. B. *Am. J. Obst. & Gynec.* (In Press.)

# NORMAL MEASUREMENTS IN RELATION TO AGE

AGE	Weight (lb.)				Height (inches)				Upper Measurement (inches)				Lower Measurement (inches)				Span (inches)			
	M.		F.		M.		F.		M.		F.		M.		F.		M.		F.	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Birth	6.2	8.6	6.4	8.6	19.3	21.1	19.0	20.8	12.1	13.1	12.1	13.1	6.9	7.9	6.9	7.9	18.1	20.1	18.1	19.9
1 Mo.	9.0	11.8	8.4	11.0	20.9	22.9	20.5	22.5	13.3	14.3	13.0	14.0	7.7	8.7	7.5	8.5	20.1	22.1	19.5	21.5
2 Mos.	10.4	13.6	9.8	12.6	22.1	24.1	21.7	23.7	13.9	14.9	13.6	14.6	8.1	9.1	7.9	8.9	21.0	23.0	20.4	22.4
3 "	11.9	15.3	11.2	14.2	23.1	25.1	22.7	24.7	14.5	15.5	14.1	15.1	8.5	9.5	8.4	9.4	22.0	24.0	21.4	23.4
4 "	13.2	16.8	12.4	15.8	24.0	26.0	23.6	25.6	15.0	16.0	14.7	15.7	9.0	10.0	8.8	9.8	22.9	25.1	22.3	24.3
5 "	13.9	17.7	13.7	17.3	24.7	26.7	24.3	26.3	15.3	16.3	15.2	16.2	9.2	10.2	9.3	10.3	23.3	25.5	23.3	25.3
6 "	15.2	19.4	14.3	18.1	25.4	27.4	25.0	27.0	15.9	16.9	15.5	16.5	9.6	10.6	9.5	10.5	24.3	26.5	23.8	25.8
7 "	15.9	20.1	14.9	18.9	26.1	28.1	25.6	27.6	16.1	17.1	15.8	16.8	9.9	10.9	9.7	10.7	24.8	27.0	24.3	26.3
8 "	16.5	20.9	15.6	19.6	26.6	28.6	26.1	28.1	16.3	17.5	16.0	17.0	10.0	11.2	10.0	11.0	25.3	27.5	24.8	26.8
9 "	17.1	21.7	16.1	20.3	27.1	29.1	26.6	28.6	16.5	17.7	16.3	17.3	10.3	11.5	10.2	11.2	25.8	28.0	25.2	27.2
10 "	17.6	22.4	16.6	21.0	27.6	29.6	27.1	29.1	16.8	18.0	16.4	17.6	10.5	11.7	10.4	11.6	26.2	28.4	25.7	27.7
11 "	18.3	23.1	17.3	21.7	28.1	30.1	27.6	29.6	17.0	18.2	16.7	17.9	10.8	12.0	10.6	11.8	26.7	28.9	26.1	28.1
12 "	18.9	23.9	17.8	22.4	28.4	30.6	28.0	30.0	17.3	18.5	16.9	18.1	11.0	12.2	10.9	12.1	27.2	29.4	26.6	28.8
15 "	20.1	25.3	18.9	23.7	29.6	31.8	29.1	31.3	17.8	19.0	17.4	18.6	11.5	12.7	11.4	12.6	28.2	30.4	27.6	29.8
18 "	21.9	27.3	20.6	25.8	30.8	33.0	30.3	32.5	18.6	19.8	18.1	19.3	12.2	13.4	12.2	13.4	29.6	32.0	29.0	31.2
21 "	23.1	28.7	21.7	27.1	31.7	34.1	31.3	33.5	18.9	20.3	18.6	19.8	12.7	14.1	12.7	13.9	30.6	33.0	29.9	32.3
24 "	24.3	30.1	22.9	28.5	32.7	35.1	32.2	34.6	19.4	20.8	18.9	20.3	13.2	14.6	13.2	14.6	31.5	33.9	30.9	33.3
30 "	26.1	32.3	24.7	30.7	34.5	36.9	33.9	36.3	20.0	21.4	19.6	21.0	14.1	15.5	14.0	15.4	32.9	35.5	32.4	34.8
36 "	28.7	35.3	26.6	33.0	36.0	38.6	35.4	38.0	20.7	22.3	20.0	21.6	15.2	16.8	14.9	16.5	34.8	37.6	33.8	36.4
42 "	30.5	37.5	28.6	35.2	37.4	40.2	36.9	39.5	21.3	22.9	20.6	22.2	16.1	17.7	15.8	17.4	36.3	39.1	35.2	38.0
48 "	31.8	39.2	30.5	37.5	38.8	41.6	38.3	40.9	21.6	23.2	21.2	22.8	16.8	18.4	16.7	18.3	37.3	40.3	36.7	39.5
54 Mos.	33.8	41.6	32.5	39.9	40.1	42.9	39.5	42.3	22.0	23.8	21.5	23.3	17.7	19.5	17.7	19.5	38.8	41.8	38.2	41.2
60 "	35.1	43.5	33.8	41.6	41.2	44.2	40.7	43.7	22.4	24.2	21.9	23.7	18.3	20.1	18.3	20.1	39.8	43.0	39.2	42.2
5½ Yrs.	37.4	46.4	35.9	44.5	42.4	45.4	41.9	44.9	22.7	24.7	22.4	24.2	19.3	21.3	19.3	21.1	41.3	44.5	40.7	43.9
6 "	39.2	48.6	37.5	46.5	43.5	46.5	43.1	46.1	23.0	25.0	22.6	24.6	20.0	22.0	19.9	21.9	42.4	45.6	41.7	44.9
6½ "	41.0	50.8	39.2	48.8	44.5	47.7	44.2	47.2	23.3	25.3	23.0	25.0	20.7	22.7	20.5	22.5	43.4	46.8	42.8	46.0
7 "	43.0	53.2	42.1	52.3	45.6	48.8	45.2	48.4	23.5	25.7	23.4	25.4	21.3	23.5	21.6	23.6	44.5	47.9	44.3	47.7
7½ "	45.0	55.8	44.1	54.9	46.6	49.8	46.3	49.5	23.8	26.0	23.7	25.9	22.0	24.2	22.1	24.3	45.5	49.1	45.4	48.8
8 "	47.2	58.4	46.4	57.6	47.5	50.9	47.2	50.6	24.1	26.3	24.0	26.2	22.7	24.9	22.8	25.0	46.8	50.4	46.5	49.9
8½ "	49.3	61.3	48.6	60.6	48.5	51.9	48.2	51.6	24.5	26.7	24.4	26.6	23.3	25.5	23.4	25.6	48.0	51.6	47.5	51.1
9 "	51.6	64.4	51.0	63.8	49.5	52.9	49.2	52.6	24.7	27.1	24.7	26.9	23.9	26.3	24.1	26.3	49.1	52.9	48.6	52.2
9½ "	54.2	67.8	53.6	67.2	50.4	54.0	50.1	53.7	25.1	27.5	25.0	27.4	24.5	26.9	24.6	27.0	50.3	54.1	49.6	53.4
10 "	57.0	71.6	56.2	71.0	51.4	55.0	51.2	54.8	25.5	27.9	25.5	27.9	25.1	27.5	25.1	27.5	51.4	55.4	50.7	54.5
10½ "	59.9	75.5	59.3	75.1	52.4	56.0	52.3	55.9	25.7	28.3	25.8	28.4	25.7	28.3	25.6	28.2	52.5	56.5	51.7	55.7
11 "	63.0	79.4	63.7	81.1	53.3	57.1	53.4	57.2	26.1	28.7	26.5	29.1	26.3	28.9	26.4	29.0	53.5	57.7	53.3	57.3
11½ "	66.2	83.2	66.9	85.5	54.3	58.1	54.6	58.4	26.5	29.1	26.9	29.5	26.9	29.5	27.0	29.6	54.6	58.8	54.2	58.4
12 "	69.4	87.2	70.7	90.5	55.2	59.0	55.6	59.6	27.0	29.6	27.4	30.0	27.4	30.0	27.5	30.1	55.8	60.0	55.4	59.6
12½ "	72.7	91.3	74.5	95.7	56.0	60.0	56.7	60.7	27.3	30.1	27.7	30.5	27.9	30.7	28.0	30.8	56.9	61.3	56.4	60.6
13 "	75.9	95.7	78.7	101.3	56.9	60.9	57.7	61.7	27.7	30.5	28.2	31.0	28.5	31.3	28.5	31.3	58.0	62.4	57.6	61.8
13½ "	79.3	100.3	83.6	107.2	57.8	61.8	58.5	62.7	28.2	31.0	28.8	31.6	29.0	31.8	28.9	31.8	59.0	63.6	58.6	63.0
14 "	81.1	102.9	89.3	113.5	58.6	62.8	59.3	63.5	28.4	31.2	29.2	32.0	29.3	32.1	29.6	32.3	59.6	64.2	59.1	63.5
14½ "	85.0	108.0	92.4	116.6	59.5	63.7	59.9	64.1	28.8	31.8	29.5	32.3	29.7	32.7	29.7	32.5	60.7	65.3	60.2	64.6
15 "	89.4	113.4	95.7	119.7	60.3	64.5	60.4	64.6	29.3	32.3	29.8	32.6	30.2	33.2	29.9	32.7	61.7	66.5	60.7	65.3
15½ "	91.6	116.2	99.0	122.8	61.1	65.3	60.8	65.0	29.5	32.5	30.1	32.9	30.5	33.5	30.1	32.9	62.3	67.1	61.3	65.9
16 "	96.0	122.0	99.0	122.8	61.8	66.2	61.1	65.3	30.0	33.0	30.1	32.9	31.0	34.0	30.1	32.9	63.4	68.2	61.3	65.9
16½ "	98.4	125.0	102.5	125.9	62.5	66.9	61.4	65.6	30.3	33.3	30.4	33.2	31.2	34.2	30.3	33.1	63.9	68.9	61.9	66.5
17 "	103.8	131.6	102.5	125.9	63.2	67.6	61.6	65.8	30.7	33.7	30.4	33.2	31.8	34.8	30.3	33.1	65.0	70.0	61.9	66.5
17½ "	106.8	135.2	106.1	128.9	63.8	68.2	61.8	66.0	31.0	34.0	30.7	33.5	32.0	35.0	30.5	33.3	65.6	70.6	62.5	67.1
18 "	109.8	139.0	106.1	128.9	64.4	68.8	61.9	66.1	31.3	34.3	"	"	32.2	35.2	"	"	66.1	71.1	62.5	67.1
18½ "	113.3	142.3	"	"	64.9	69.3	"	"	31.6	34.6	"	"	32.4	35.4	"	"	66.6	71.8	"	"
19 "	117.2	145.6	"	"	65.3	69.7	"	"	31.9	34.9	"	"	32.6	35.6	"	"	67.2	72.4	"	"
19½ "	121.5	148.5	"	"	65.6	70.0	"	"	32.1	35.3	"	"	32.7	35.9	"	"	67.8	73.0	"	"
20 "	121.5	148.5	"	"	65.8	70.2	"	"	32.1	35.3	"	"	32.7	35.9	"	"	67.8	73.0	"	"

FIG. 49.

AVERAGE WEIGHT FOR WOMEN BASED ON HEIGHT AND AGE.

Height (Inches)	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	Height (Inches)
Age																		Age
20	106	108	110	112	114	116	119	122	125	128	132	136	140	143	147	151	156	20
21	107	109	111	113	115	117	120	123	126	129	133	137	141	144	148	152	156	21
22	107	109	111	113	115	117	120	123	126	129	133	137	141	145	149	153	157	22
23	108	110	112	114	116	118	121	124	127	130	134	138	142	146	150	154	158	23
24	109	111	113	115	117	119	121	124	127	130	134	138	142	146	150	154	158	24
25	109	111	113	115	117	119	121	124	128	131	135	139	143	147	151	154	158	25
26	110	112	114	116	118	120	122	125	128	131	135	139	143	147	151	155	159	26
27	110	112	114	116	118	120	122	125	129	132	136	140	144	148	152	155	159	27
28	111	113	115	117	119	121	123	126	130	133	137	141	145	149	153	156	160	28
29	111	113	115	117	119	121	123	126	130	133	137	141	145	149	153	156	160	29
30	112	114	116	118	120	122	124	127	131	134	138	142	146	150	154	157	161	30
31	113	115	117	119	121	123	125	128	132	135	139	143	147	151	154	157	161	31
32	113	115	117	119	121	123	125	128	132	136	140	144	148	152	155	158	162	32
33	114	116	118	120	122	124	126	129	133	137	141	145	149	153	156	159	162	33
34	115	117	119	121	123	125	127	130	134	138	142	146	150	154	157	160	163	34
35	115	117	119	121	123	125	127	130	134	138	142	146	150	154	157	160	163	35
36	116	118	120	122	124	126	128	131	135	139	143	147	151	155	158	161	164	36
37	116	118	120	122	124	126	129	133	136	140	144	148	152	156	159	162	165	37
38	117	119	121	123	125	127	130	133	137	141	145	149	153	157	160	163	166	38
39	118	120	122	124	126	128	131	134	138	142	146	150	154	158	161	164	167	39
40	119	121	123	125	127	129	132	135	138	142	146	150	154	158	161	164	167	40
41	120	122	124	126	128	130	133	136	139	142	147	151	155	159	162	165	168	41
42	120	122	124	126	128	130	133	136	139	143	147	151	155	159	162	165	168	42
43	121	123	125	127	129	131	134	137	140	144	148	152	156	160	163	167	170	43
44	122	124	126	128	130	132	135	138	141	145	149	153	157	161	164	168	171	44
45	122	124	126	128	130	132	135	138	141	145	149	153	157	161	164	168	171	45
46	123	125	127	129	131	133	136	139	142	146	150	154	158	162	165	169	172	46
47	123	125	127	129	131	133	136	139	142	146	151	155	159	163	166	170	173	47
48	124	126	128	130	132	134	137	140	143	147	152	156	160	164	167	171	174	48
49	124	126	128	130	132	134	137	140	143	147	152	156	161	165	168	172	175	49
50	125	127	129	131	133	135	138	141	144	148	152	156	161	165	169	173	176	50
51	125	127	129	131	133	135	138	141	144	148	152	157	162	166	170	174	177	51
52	125	127	129	131	133	135	138	141	144	148	152	157	162	166	170	174	177	52
53	125	127	129	131	133	135	138	141	144	148	152	157	162	166	170	174	177	53
54	125	127	129	131	133	135	138	141	144	148	153	158	163	167	171	174	177	54
55	125	127	129	131	133	135	138	141	144	148	153	158	163	167	171	174	177	55

FIG. 50

(From "Endocrinology in Women", by Dr. E. C. Hamblen, published by Chas. C. Thomas Co., by kind permission of author and publisher)



# APPEARANCE OF OSSEOUS CENTRES AND EPIPHYSEAL CLOSURE

Age in Years	Wrist A.P.	Elbow A.P.	Elbow Lat.	Ankle A.P.	Ankle Lat.	Hips A.P.
Birth					Talus Cuboid Calcaneum	
1	Capitate Hamate Epiph. lower end of Radius				Lat. cuneiform Epiph. lower end of Tibia	Epiph. of head of Femur
2		Capitulum of Humerus			Epiph. lower end of Fibula	
3	Triquetrum Epiph. Phalanges and Metacarpals				Epiphyses Metatarsals medial Cuneiform	
4	Lunate				Intermediate cuneiform Navicular	Epiph. of great Trochanter
5	Trapezium Scaphoid	Epiph. of head of Radius				
6	Epiph. lower end of Ulna	Medial epicondyle of Humerus				
7						Union of ischium and pubis
8					Epiph. of Calcaneum	
9			Trochlea Olecranon			
10	Pisiform					Epiph. of lesser Trochanter of Femur
11		Lateral epicondyle of Humerus				
12		Union of trochlea and capitulum				
13-14			Olecranon			
14-15		Head of Radius, lat. epicondyle of Humerus			Epiph. of Calcaneum	
15-16	Epiphyses of Phalanges and Metacarpals			Epiphyses of Phalanges and Metatarsals		Trochanters: Head of Femur
17-19				Epiphyses lower end of Tibia and Fibula		
18-20	Epiphyses of lower end of Radius and Ulna					

FIG. 51



*Male 9 months*



*Female 9 months*



*Male 21 months*



*Female 21 months*

**FIG. 52.—RADIOGRAPHS OF OSSEOUS MATURATION**

(Figs. 52-57 from T. Wingate Todd's "Atlas of Skeletal Maturation", by kind permission of the publishers, C. V. Mosby Co.)





*Male 2 years 9 months*



*Female 2 years 9 months*



*Male 3 years 3 months*



*Female 3 years 3 months*

FIG. 53.—RADIOGRAPHS OF OSSEOUS MATURATION





*Male 4 years 3 months*



*Female 4 years 3 months*



*Male 5 years 3 months*



*Female 5 years 3 months*

FIG. 54.—RADIOGRAPHS OF OSSEOUS MATURATION



*Male 6 years 3 months*



*Female 6 years 3 months*



*Male 7 years 9 months*



*Female 7 years 9 months*

FIG. 55.—RADIOGRAPHS OF OSSEOUS MATURATION





*Male 8 years 9 months*



*Female 8 years 9 months*



*Male 13 years 9 months*



*Female 13 years 9 months*

FIG. 56.—RADIOGRAPHS OF OSSEOUS MATURATION





*Male 16 years 9 months*



*Male 18 years 9 months*



*Female 14 years 9 months*



*Female 15 years 9 months*

## COMMERCIAL PREPARATIONS\*

THE preparations listed are intended as a general guide. The list does not aim at completeness and omission of a preparation does not imply that it is less effective than those mentioned.

Name of Preparation	Description	Maker
ANTERIOR PITUITARY EXTRACT FROM THE GLAND		
<i>Total Gland Preparations</i>		
Preloban	Each ampoule contains 25 maturation units, with solvent	Bayer (B)
Polyansyn	Each 5-c.c. vial contains total hormone of anterior pituitary	Armour
Pituitary Gona- dotrophin (of equine pitui- tary origin)	1 c.c. contains 25 r.u.	
<i>Growth Hormone</i>		
Antuitrin "S"	Rubber-capped vials of 20 c.c. 1 c.c. contains 10 rat units	Parke Davis
Phykentrone	1 c.c. contains 20 growth units	Squibb (USA)
<i>Thyrotrophic Hormone</i>		
Thyrogan	Each ampoule contains 50 guinea-pig weight units, with ampoules of solvent	B.D.H. (B)
Thyrotropin	Each ampoule contains 100 guinea-pig units of hormone from approximately 1 gram fresh anterior pituitary, with solvent	Paines & Byrne (B)
Thyrotropic Factor	1 c.c. contains 5 Rowland-Parkes units	Armour (USA)
Thyrotropic Factor	1 c.c. contains 50 hypophysectomized rat units	Ayerst (USA)

\* All these preparations are available either in Great Britain or in the United States and many are available in both. Preparations available only in the former are marked (B); those available only in the latter are marked (USA); preparations not so marked are obtainable in both.

Name of Preparation	Description	Maker
<i>Corticotrophic Hormone</i>		
Cortrophin	Each ampoule contains 5 and 10 Sudanophobic units, with ampoules of solvent	Organon (B)
Adrenotropic Factor	1 c.c. contains 10 hypophysectomized rat units	Armour (USA)

*Lactogenic Hormone*

Physolactin	1 c.c. contains not less than 60 Riddle-Bates units	Glaxo (B)
Prolactin	1 c.c. contains 100 Prolactin units (Riddle)	Armour
Prolactin	1 c.c. contains 60 Riddle units	A. & H. (B)
Luteotrophin	1 c.c. contains 200 I.U.	Squibb (USA)
Prolactin	Each ampoule contains 100 I.N.	Schering (USA)

*Combined Hormones*

Ambinon "A"	Each ampoule contains 100-300 guinea-pig units thyrotrophic hormone, 50 rat units pituitary gonadotrophic hormone, with 100 rat units chorionic gonadotrophin	Organon (B)
Synapoidin	Contains anterior pituitary FSH hormone and chorionic gonadotrophic hormone. 10-c.c. vials, each 1 c.c. represents 15 synergy rat units	Parke Davis
Ambinon "B"	Each ampoule contains 100-300 guinea-pig units thyrotrophic hormone, with traces of other anterior pituitary hormones	Organon (B)

## GONADOTROPHIC HORMONE FROM PREGNANT MARES' SERUM

Antostab	Each ampoule contains 100 mouse units (equivalent to 200 rat vaginal cornification units) with solvent	Boots (B)
Gestyl	Each ampoule contains 200, 400, 1,000 or 3,000 I.U. of serum gonadotrophin	Organon (B)
Gonadyl	Each ampoule contains 40 Evans' Units (400 mouse units), with solvent	Roussel (B)



Name of Preparation	Description	Maker
Serogan	Each ampoule contains 200 or 1,000 I.U. together with ampoules of solvent	B.D.H. (B)
Anteron	Each vial contains 5,000 I.N.	Schering (USA)
Gonadogen	Each vial contains 10-20 Cartland-Nelson units (approximately 200 or 400 I.N.)	Upjohn (USA)
Antex	1 c.c. contains 500 or 1,000 I.N.	Ayerst (USA)

### GONADOTROPHIC HORMONE FROM PREGNANCY URINE

Antuitrin "S" (Antroidin)	10-c.c. vials, 100 I.U. per c.c., and 5-c.c. vials, 500 I.U. per c.c.	Parke Davis
Follutein	Each vial contains 500, 1,000 or 5,000 rat units	Squibb (B)
Follutein	1 c.c. contains 100, 500 or 1,000 I.U.	Squibb (USA)
Gonan	Each ampoule contains 100 or 500 I.U. with solvent	B.D.H.
Physostab	Each ampoule contains 100 mouse units (equivalent to 1,500 rat vaginal cornification units), with solvent	Boots (B)
Pregnyl	Each ampoule contains 100, 500 or 1,500 I.U. with solvent	Organon
Prolan	Each ampoule contains 100 or 2,000 I.U. with solvent	Bayer (B)
Pranturon	Each vial contains 5,000 or 1,000 I.U.	Schering (USA)
A.P.L.	1 c.c. contains 100, 500 or 1,000 I.U.	Ayerst (USA)

### POSTERIOR PITUITARY GLAND HORMONE

Pitocin	(Oxytocic) Ampoules of 0.5 c.c. and 1 c.c. (1 c.c. represents 10 I.U.)	Parke Davis
Pitressin	(Pressor and anti-diuretic.) Ampoules of 0.5 c.c. and 1 c.c. (1 c.c. represents 10 or 20 pressor units.)	Parke Davis
Pitressin Tannate in oil	1 c.c. represents 5 pressor units	Parke Davis

Name of Preparation	Description	Maker
Pituitrin	(Pressor, oxytocic and anti-diuretic.) Surgical ampoules of 0.5 c.c. and 1 c.c. (1 c.c. represents 20 I.U.) Obstetrical ampoules of 0.5 c.c. and 1 c.c. (1 c.c. represents 10 I.U.)	Parke Davis
Pituitary (Posterior) Desiccated Powder	Phials of 1 drachm for intranasal insufflation	Armour
Pitone Snuff	In tubes of 2 grams 500 I.U. Posterior pituitary extract per 1 gram in lactose	Organon

#### THYROID GLAND HORMONE

Desiccated Thyroid Tablets	$\frac{1}{10}$ - 5 grains or 5-100 mg. (0.75-1.5 grains) The B.P. product contains 0.09-0.11 per cent of iodine in combination as thyroxin The U.S.P. product contains 0.17-0.23 per cent of iodine	
Thyroxin sodium B.P.	Contains 61-4 per cent of iodine 1 mg. is analogous in action to 0.2 gram of the dried gland and causes an average increase of 2.8 per cent in the B.M.R. <i>Tablets</i> are graded in mg. <i>Ampoules</i> 1-10 mg. of sterile crystals or solution of 2 mg. per c.c. (Both are for intravenous injection)	
Thyroxin U.S.P.	Contains not less than 64 per cent of iodine in ampoules and tablets	

#### IRRADIATED ERGOSTEROL PREPARATIONS

Dihydrotachysterol. Syn. A.T. 10	In oil solution 0.5 per cent. Does not contain Vitamin D	Savory and Moore (B)
Radiostol (Calciferol)	1 mg. equals 40,000 I.U. vitamin D Pellets: 1 pellet contains 3,000 I.U. Solution: 1 oz. with a dropper contains 3,000 I.U. in 1 gram of solution Solution: 100,000 I.U. per gram Solution: 200,000 I.U. per gram	B.D.H. (B)

Name of Preparation	Description	Maker
Ostelin	Tablets: 1 tablet contains 500 I.U. vitamin D Solution: 1 c.c. contains 5,000 I.U. vitamin D	Glaxo (B)
Ostelin High Potency	Tablets: 1 tablet contains 50,000 I.U. vitamin D Ampoules: 1 c.c. contains 300,000 I.U. vitamin D	Glaxo (B)

## PARATHYROID HORMONE

Para-Thor-Mone	5-c.c. rubber-capped vials and 1-c.c. ampoules for subcutaneous, intramuscular or intravenous injection. (1 c.c. contains 20 units)	Lilly
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## ADRENAL CORTICAL HORMONE

Cortin	10-c.c. vials aqueous solution for intramuscular and intravenous injection. 1 c.c. represents 30 grams of Cortex or 50 grams of the whole gland	Organon
Eschatin	1 c.c. represents 40 grams of Cortex or 25 dog units	Parke Davis
Eucortone	1 c.c. represents 75 grams of Cortex or 110 grams of the whole gland	A. & H.
Doca (Desoxy-corticosterone acetate)	Ampoules of 1 c.c. contain 2 mg., 5 mg., or 10 mg. Rubber-capped vials of 10 c.c. contain 5 mg. per c.c.	Organon (B) Roche-Organon (USA)
	Pellets for implantation 25, 50, 100, 150, 200, 300 mg.	Organon (B)
Cortate	Pellets containing 75 mg. of desoxy-corticosterone acetate in individual sterile vials. Boxes of 1 and 3 vials	Schering (USA)
Percorten	Ampoules of 1 c.c. contain 2 mg., 5 mg., or 10 mg.	Ciba



Name of Preparation	Description	Maker
INSULIN PREPARATIONS		
Soluble Insulin	In 5- or 10-c.c. rubber-capped vials "U-20", "U-40", "U-80" and so forth, indicating the number of units contained in 1 c.c.	B. Wellcome (B), B.D.H. (B), A. & H. (B)
Iletin	In 10-c.c. vials, designated as U-40 and U-80 containing 40 and 80 units per c.c. respectively	Lilly (SA)
Iletin made from zinc-insulin crystals	is supplied in 10-c.c. vials designated U-40 and U-80 containing 40 and 80 units per c.c. respectively	Lilly (USA)
Insulin Squibb	is supplied in 10-c.c. vials containing 40 and 80 units per c.c. respectively	Squibb (USA)
Insulin from zinc-Insulin Crystals	10-c.c. vials containing 40 and 80 units per c.c. respectively	Squibb (USA)
Protamine Zinc Insulin	Vials containing 40 or 80 units	B. Wellcome (B), B.D.H. (B), A. & H. (B)
Protamine, Zinc and Iletin	is supplied in 10-c.c. vials containing 400 and 800 units, labelled 40 and 80 units per c.c. respectively	Lilly (USA)
Insulin Protamine Zinc	In 10-c.c. vials containing 40 and 80 units per c.c. respectively	Squibb (USA)
Globin Zinc Insulin	5-c.c. vials (40 units per c.c.). 5-c.c. vials (80 units per c.c.)	B. Wellcome (B), B.D.H. (B), A. & H. (B)
Globin Insulin with Zinc	Available in 40 and 80 units per c.c., vials of 10 c.c.	B. Wellcome (USA)
Insulin-Globin with Zinc	In 10-c.c. vials containing 40 and 80 units per c.c. respectively	Squibb (USA)
Insulin Powder	10 I.U. per 1 gram	Organon (B)
Insulin Ointment	1 I.U. and 10 I.U. per 1 gram	Organon (B)

Name of Preparation	Description	Maker
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## OESTROGENIC PREPARATIONS

*Oestrone Ampoules*

Amniotin	1 c.c. contains 2,000 or 10,000 I.U.	Squibb
Menformon	Aqueous solution for intravenous and intramuscular injection. 1 c.c. contains 100 or 1,000 I.U. Oil solution for intramuscular injection only. 1 c.c. contains 10,000 I.U. Also 5-c.c. vials	Organon
Oestroglandol	1 c.c. contains 1,000 I.U.	Roche (B)
Oestrone	1 c.c. contains 10,000 or 50,000 I.U.	Ciba
Theelin (Aqueous)	1 c.c. contains 200 I.U.	Parke Davis
Theelin in Oil	1 c.c. contains 1,000, 2,000, 5,000 or 10,000 I.U.	Parke Davis
Unden	1 c.c. contains 1,000 I.U.	Bayer (B)
Estrone	1 c.c. contains 0.1, 0.2, 0.5 and 1.0 mg. (1,000, 2,000, 5,000 and 10,000 I.U.)	Lilly (USA)
Estrone, Aqueous suspension	1 c.c. contains not less than 2 mg. (2,000 I.U.)	Lilly (USA)

*Oestrone Capsules and Tablets*

Amniotin Capsules	Each capsule contains 1,000 or 2,000 I.U.	Squibb
Menformon Tablets	Each tablet contains 100, 500, 1,000, 3,000, 10,000 or 50,000 I.U.	Organon
Oestroglandol	Each tablet contains 1,000 I.U.	Roche (B)
Ovostab Tablets	Each tablet contains 1,000 or 10,000 I.U.	Boots (B)
Progynon Tablets	Each tablet contains 1,000, 3,000 or 10,000 I.U.	Schering (B)
Premarin	Sodium oestrone sulphate obtained from pregnant mares' urine. Each tablet contains 0.625 and 1.25 mg.	Ayerst (USA)

*Oestrone Preparations*

Amniotin Pessaries	Each pessary contains 1,000 or 2,000 I.U.	Squibb
Amniotin for Nasal Use	10,000 I.U. per c.c. or 20,000 I.U. in 30 c.c.	Squibb
Kolpon Vaginal Bougies	Each bougie contains 500 I.U.	Organon (B)

Name of Preparation	Description	Maker
Kolpon Vaginal Tablets	Buffered Glucose Tablets. Each tablet contains 1,000 I.U.	Organon (B)
Kolpon Inserts	Each tablet-suppository contains 500 I.U. (children's size) and 1,000 I.U. (adult size)	Roche-Organon (USA)
Menformon Drops	1 c.c. contains 10,000 I.U. in oil	Organon
Menformon Ointment	1 gram. contains 5,000 I.U.	
Menformon Suppositories	Each suppository contains 1,000 or 10,000 I.U.	Organon
Oestroglandol Ointment	1 gram contains 1,000 I.U.	Roche (B)
Theelin Vaginal Suppositories	Each vaginal suppository contains 2,000 I.U.	Parke Davis
Theelin in Oil for Nasal Use	1 c.c. contains 1,000 I.U.	Parke Davis
Unden Ointment	1 gram contains 5,000 I.U.	Bayer (B)
<i>Oestradiol Ampoules</i>		
Benzo-Gynoestryl	1 c.c. contains 1,000, 10,000 or 50,000 I.B.U.	Roussel (B)
Di-Menformon	1 c.c. contains 10,000 or 50,000 I.B.U. Also 5- and 10-c.c. vials	Organon (B)
Dimenformon Benzoate	1 c.c. contains 0.1, 0.166, 0.33 and 1.0 mg. (600, 1,000, 2,000 and 6,000 r.u.)	Roche-Organon (USA)
Oestroform	1 c.c. contains 1,000, 10,000, 20,000 or 50,000 I.B.U.	B.D.H. (B)
Ovostab	1 c.c. contains 2,000, 10,000 or 50,000 I.B.U.	Boots (B)
Progynon B. Oleosum	1 c.c. contains 10,000 or 50,000 I.B.U.	Schering (B)
Progynon-B	1 c.c. contains 500, 1,000, 2,000 and 10,000 r.u. (0.083, 0.166, 0.333, 1.0 and 1.666 mg.)	Schering (USA)
Ben-Ovocylin	1 c.c. contains 0.1666, 0.333, 1.0 and 1.66 mg. (1,000, 2,000, 6,000 and 10,000 r.u.)	Ciba (USA)
Lynoral	Each tablet contains 0.05 mg.	Roche-Organon (USA)



Name of Preparation	Description	Maker
<i>Oestradiol Tablets</i>		
Gynoestryl	Each tablet contains 0.025 mg.	Roussel (B)
Gynöstrol S	Each tablet contains 0.2 mg.	Roussel (B)
Oestroform	Each tablet contains 1,000, 5,000 or 10,000 I.U.	B.D.H. (B)
Ovocyclin	Each tablet contains 0.04 mg. or 0.2 mg.	Ciba (B)
Ovocylin	Each tablet contains 0.1, 0.2 and 0.5 mg.	Ciba (USA)
Progynon-DH	Each tablet contains 1,200, 2,400 and 6,000 r.u. (0.1, 0.2 and 0.5 mg.)	Schering (USA)
Dimenformon	Each tablet contains 0.1, 0.2 and 0.5 mg. (1,200, 2,400, 6,000 r.u.)	Roche-Organon (USA)

*Oestradiol Dipropionate*

Ovocyclin P	1 c.c. contains 10,000 or 50,000 "dipropionate units"	Ciba (B)
Di-Ovocylin	1 c.c. contains 0.1 mg., 0.2 mg. and 5.0 mg.	Ciba (USA)
Progynon D.P.	1 c.c. contains 20,000 oestradiol dipropionate units 1 c.c. contains 0.2, 0.5, 1.0 and 5 mg.	Schering Schering (USA)
Dimenformon Dipropionate	1 c.c. contains 5 mg. 5-c.c. vials	Organon
Ethinyl Estradiol	Each tablet contains 0.02 mg. and 0.05 mg.	Schering (USA)
Ethinyl Estradiol-Ciba	Each tablet contains 0.02 and 0.05 mg.	Ciba (USA)

*Oestradiol and Benzoate Preparations*

Di-Menformon Ointment	1 gram contains 20,000 I.B.U. oestradiol benzoate	Organon
Ecto-Gynoestryl Ointment	25 grams contains 2.5 mg. oestradiol	Roussel (B)
Ecto-Gynoestryl Solution	10 c.c. contains 5 mg. oestradiol	Roussel (B)
Gynoestryl Solution for Oral Use	10 c.c. contains 1 mg. oestradiol	Roussel (B)
Oestroform Pessaries	Each pessary contains 1,000 I.U.	B.D.H. (B)

Name of Preparation	Description	Maker
Ovocyclin Ointment	25 grams contains 2.5 mg. oestradiol	Ciba
Progynon Ointment	25 grams contains 2.5 mg. oestradiol in neutral base	Schering (B)
Progynon-DH	1 gram contains 0.03 mg. or 0.15 mg.	Schering (USA)
Progynon Vaginal Capsules	Each capsule contains 0.25 mg. oestradiol	Schering (B)
Progynon Vaginal Suppositories	Each suppository contains 0.36 mg. oestradiol	Schering (B)
Progynon-DH	Juvenile size. Each containing 480 r.u. (0.04 mg.) Adult size. Each containing 480 and 4,800 r.u. (0.04 and 0.4 mg.)	Schering (USA)
Sedo-Gynoestryl	1 c.c. contains 1,000 I.U. oestradiol, with 0.5 gram sodium bromide and 0.02 gram Ext. Hyoscy.	Roussel (B)
<i>Oestriol</i>		
Emmenoplex	1 c.c. contains 30 (Wistar) units oestriol monoglycuronide	Glaxo (B)
Theelol Capsules	Each capsule contains 200 or 400 I.U.	Parke Davis
<i>Stilboestrol Ampoules</i>		
Stilboestrol-Boots	1 c.c. contains 1 or 5 mg.	Boots (B)
Stilboestrol-B.D.H.	1 c.c. contains 1 or 5 mg.	B.D.H. (B)
Stilboestrol Hypoloid	1 c.c. contains 1 or 5 mg.	B. Wellcome (B)
Diethylstilbestrol	1 c.c. contains 0.25, 0.5, 1.0 and 5.0 mg.	Lilly (USA)
<i>Stilboestrol Tablets</i>		
Clinestrol	Each tablet contains 0.5, 1 or 5 mg.	Glaxo (B)
Stilboestrol-A. & H.	Each tablet contains 0.5, 1 or 5 mg.	A. & H. (B)
Stilboestrol-Boots	Each tablet contains 0.1, 0.5, 1.0 or 5 mg.	Boots (B)
Stilboestrol-B.D.H.	Each tablet contains 0.5, 1 or 5 mg.	B.D.H. (B)
Stilboestrol-Organon	Each tablet contains 0.1, 0.5, 1 or 5 mg.	Organon (B)
Stilboestrol-Tabloid	Each tablet contains 0.25, 0.5, 1 or 5 mg.	B. Wellcome (B)

Name of Preparation	Description	Maker
Diethylstilbestrol	Each tablet contains 0.1, 0.25, 0.5, 1 and 5 mg.	Lilly (USA) Squibb (USA)
<i>Stilboestrol Preparations</i>		
Neo-oestranol-1 (Nasal Spray)	1 c.c. contains 0.1 mg.	Crookes (B)
Neo-oestranol-1 Salve	1 gram contains 0.1 mg.	Crookes (B)
Neo-oestranol-1 Pessaries	Each pessary contains 0.25 mg.	Crookes (B)
Pabestrol Sup- positories	Each suppository contains 2 mg.	Paines & Byrne (B)
Pabestrosalve	1 gram contains 1 mg.	Paines & Byrne (B)
Wellcome Stilb- oestrol Oint- ment	Each gram contains $\frac{1}{2}$ mg. of stilb- oestrol dipropionate	B. Wellcome (B)
<i>Stilboestrol Dipropionate Ampoules</i>		
Clinestrol	1 c.c. contains 1 or 5 mg.	Glaxo (B)
Stilboestrol Di- propionate- Boots	1 c.c. contains 1 or 5 mg.	Boots (B), Organon (B)
Stilboestrol Di- propionate Hypoid	1 c.c. contains 1 or 5 mg.	B. Wellcome (B)
Stilboestrol Di- propionate- B.D.H.	1 c.c. contains 1 or 5 mg.	B.D.H. (B)
Diethylstilbestrol	Each ampoule contains 0.2, 0.5, 1 and 5 mg.	Squibb (USA)
<i>Stilboestrol Dipropionate Tablets</i>		
Stilboestrol Di- propionate- Boots	Each tablet contains 0.1, 0.5, 1 or 5 mg.	Boots (B)
Stilboestrol Di- propionate- B.D.H.	Each tablet contains 0.5, 1 or 5 mg.	B.D.H. (B)
Stilboestrol Di- propionate- Tabloid	Each tablet contains 1 or 5 mg.	B. Wellcome (B)
Diethylstilbestrol	Each tablet contains 0.1, 0.25, 0.5, 1.0 and 5.0 mg.	Lilly (USA)



Name of Preparation	Description	Maker
<i>Hexoestrol Ampoules</i>		
Hexoestrol	1 c.c. contains 1 or 5 mg.	B.D.H. (B)
Hexoestrol	1 c.c. contains 1 or 5 mg.	B. Wellcome (B)
Synthovo	1 c.c. contains 1 or 5 mg.	Boots (B)
<i>Hexoestrol Tablets</i>		
Hexoestrol	Each tablet contains 0.5, 1 or 5 mg.	B.D.H. (B)
Hexoestrol-Organon	Each tablet contains 0.1, 0.5, 1 or 5 mg.	Organon (B)
Hexoestrol	Each tablet contains 1 or 5 mg.	B. Wellcome (B)
Synthovo	Each tablet contains 1 or 5 mg.	Boots (B)
Hexestrol	Each tablet contains 1.0 and 3.0 mg.	Ortho(USA)
Hexestrol	Each tablet contains 0.2, 1.0 and 3.0 mg.	Merrell (USA)
<i>Dienoestrol Tablets</i>	Each tablet contains 0.1, 0.3, 1 or 5 mg.	B.D.H. (B), Glaxo (B)
	Each tablet contains 0.03, 0.1, 0.3, 1 or 5 mg.	Organon (B)
<i>Octofollin (Benzoestrol)</i>	Not available in the U.K.	
Benzestrol	Dispensed in vials containing 5 mg. per c.c. Tablets containing 0.5, 1, 2 and 5 mg.	Schieffelin (USA)

## PROGESTOGENIC PREPARATIONS

*Progesterone Ampoules*

Lipo Lutin	1 c.c. contains 1 or 2 mg.	Parke Davis
Luteostab	1 c.c. contains 2 or 5 mg.	Boots (B)
Lutocyclin	1 c.c. contains 2, 5 or 10 mg.	Ciba (B)
Lutocylin	1 c.c. contains 2, 5 or 10 mg.	Ciba (USA)
Lutogyl	1 c.c. contains 2 mg., 5 mg. or 10 mg.	Roussel (B)
Progesterone	1 c.c. contains 1 mg., 2 mg. or 5 mg.	Oxo (B)
Progestin-Organon	1 c.c. contains 1 mg., 2 mg., 5 mg. or 10 mg. Also in 5-c.c. phials and 1 mg. per 1 c.c. in 10-c.c. phials	Organon
Progestin-B.D.H.	1 c.c. contains 1 mg., 2 mg. or 5 mg.	B.D.H. (B)
Proluton	1 c.c. contains 2 mg., 5 mg. or 10 mg.	Schering

*Pellets for Implantation*

Progesterone	25 mg., 50 mg. and 100 mg.	Organon
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Name of Preparation	Description	Maker
<i>Anhydro-hydroxy-progesterone Tablets</i>		
Lutocyclin	Each tablet contains 5 or 10 mg. anhydro-hydroxy-progesterone	Ciba (B)
Lutocylol	Each tablet contains 5 or 10 mg. anhydro-hydroxy-progesterone	Ciba (USA)
Lutogyl	Each tablet contains 5 or 10 mg. synthetic progesterone	Roussel (B)
Progestoral	Each tablet contains 5 or 10 mg. ethisterone	Organon
Proluton C	Each tablet contains 5 or 10 mg. anhydro-hydroxy-progesterone	Schering (B)
Pranone	Each tablet contains 5 or 10 mg. anhydro-hydroxy-progesterone	Schering (USA)

## ANDROGENIC PREPARATIONS

*Androsterone Ampoules*

Erugon	1 c.c. contains 2 comb-growth units	Bayer (B)
Proviron	1 c.c. contains 5 mg. of androsterone benzoate	Schering (B)

*Testosterone Propionate Ampoules*

Neo-Hombreol	1 c.c. contains 5, 10 or 25 mg.	Organon
Perandren	1 c.c. contains 5, 10 or 25 mg.	Ciba
Sterandryl	1 c.c. contains 5, 10 or 25 mg.	Roussel (B)
Testoviron	1 c.c. contains 5, 10 or 25 mg.	Schering (B)
Oreton	1 c.c. contains 5, 10 or 25 mg.	Schering (USA)

*Testosterone Propionate Ointment and Solution for External Use*

Ecto-Sterandryl	10 c.c. contains 50 mg.	Roussel (B)
Neo-Hombreol Ointment (weak)	1 gram contains 2 mg.	Organon
Neo-Hombreol Ointment (strong)	1 gram contains 25 mg.	Organon
Perandren Ointment	25 grams contains 50 mg. as testosterone	Ciba
Testoviron Ointment	25 grams contains 50 mg. (base)	Schering (B)

Name of Preparation	Description	Maker
<i>Testosterone Pellets</i>		
Oreton-F	Chemically pure crystalline pellets for implantation. 25, 50, 100, 150, 200, 300 and 350 mg. 75 mg.	Organon (B) Schering (USA)

*Methyl testosterone*

Neo-Hombreol (M)	Each tablet contains 5 or 10 mg. of methyl testosterone	Organon
Methyl Testosterone, Boots	Each tablet contains 5 mg.	Boots (B)
Metandren	Each tablet contains 5 mg.	Ciba
Oreton-M	Each tablet contains 10 mg.	Schering (USA)
Methyl Testosterone Ointment	Special, sealed, gelatin capsules, each containing 2 grams of ointment in which 4 mg. of methyl testosterone is incorporated	Roche- Organon (USA)
Neo-Hombreol (M) Dosules		
Oreton-M	4 mg. per 2½ inches of ribbon as squeezed from tube	Schering (USA)

*Testosterone Preparations*

Neo-Hombreol Suppositories	Each suppository contains 15 mg.	Organon
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## • THIOURACIL PREPARATIONS

Thiouracil-B.D.H.	Each tablet contains 0.05, 0.1 and 0.2 gram	B.D.H. (B)
2-Thiouracil (M. & B.)	Each tablet contains 0.05, 0.1 and 0.2 gram	May & Baker (B)
Thiouracil (Organon)	Each tablet contains 50 and 100 mg.	Organon (B)
Methyl Thiouracil	Each tablet contains 50 and 200 mg.	Organon (B)
Thiouracil	Each tablet contains 0.1 and 0.2 gram	Lilly (USA), Squibb (USA), Parke Davis
Propylthiouracil	Each tablet contains 50 mg.	Lilly (USA)



# INDEX



# INDEX

## A

- Abortion, habitual, threatened, treatment with thyroid hormone, 53
- prevention of, by progestin, 172
- threatened, treatment with progestogens, 177
- — — — vitamin E, 244
- treatment with progestogens, 177
- Acetylcholine, 82
- Achard-Thiers syndrome, 122
- Acidophil cells, 1
- Acne, treatment of, with insulin, 130
- Acromegaly, 28
- thymus hyperplasia in, 206
- treatment of, 5
- — with oestrogens, 162
- Adams-Stokes syndrome, treatment of, with adrenalin, 109
- Adaptation syndrome, preventive rôle of adrenals in, 82
- Addison's disease, 122
- — diagnosis, water test for, 288
- — effect on electroencephalogram, 220
- — severe crisis in, treatment of, 105
- — treatment with adrenal hormones, 105
- — — — androgens, 194
- Adrenal cortex, 68
- — and vitamins, 100
- — autonomy of, 8
- — carcinoma of, 79
- — corticotrophic factors, 85
- — effect of alarm reaction on, 83
- — — — insulin on, 9
- — — — progesterone on, 173
- — — — thyroid hormone on, 47
- — — on basal metabolism, 77
- — — — carbohydrate metabolism, 76
- — — — fat metabolism, 77
- — — — gonadal system, 78
- — — — growth, 78
- — — — haemopoietic system, 77
- — — — protein metabolism, 76
- — extract, clinical applications of, 105
- Adrenal cortex extracts, 70
- — — bovine, effects of, 103
- — — porcine, effects of, 103
- — — preparations for clinical use, 103, 359
- — — standardization of, 101
- — hormone of, 7
- — — assay methods, 284
- — — commercial preparations of, 359
- — hyperfunction, 73
- — — effects of, 89
- — hyperplasia, effects of, 78
- — hypertrophy of, compensatory, 72
- — insufficiency, 73
- — — chronic, symptoms of, 78
- — — effects of, 75, 88
- — physiology of, 71
- — production of androgens by, 180
- — — — oestrogens by, 137
- — protective effects, 80
- — relation to other endocrine glands, 94
- — — — parathyroid, 60, 97
- — — — posterior pituitary, 39, 96
- — — — thyroid hormone, 9, 96
- — — — vitamin B<sub>1</sub>, 238
- — rôle in lactation, 80, 100
- — tumours of, signs and symptoms, 78
- gland, effect of cold on, 210
- — hormones, 68, 117
- — relationship to androgens, 188
- — — — thymus, 206
- — visualization of, by perirenal insufflation, 290
- — vitamin C storage in, 242
- insufficiency, acute, in children, treatment of, 106
- — chronic, treatment of, 106
- medulla, 89
- and vitamins, 100
- effect of parathyroid on, 60
- effects, summary of, 94
- physiology of, 90
- relation to other endocrine glands, 94



- Adrenal medulla, relation to posterior pituitary, 39  
 — — — — thyroid gland, 48  
 — — secretion of adrenalin by, 90  
 Adrenalectomy, effects of, 80  
 Adrenalin, 90  
 — clinical applications of, 108  
 — effect on insulin, 123  
 — — — muscular system, 91  
 — — — nervous system, 90  
 — — — oestrogens, 146  
 — effects on basal metabolism, 92  
 — — — blood-vessels, 91  
 — — — carbohydrate metabolism, 92  
 — — — glands, 92  
 — preparations of, for clinical use, 104  
 — protective effects of, 93  
 — secretion, control of, 93  
 — — stimulating factors, 93  
 — standardization of, 102  
 — toxic effects of, 93  
 Adrenocorticotrophic hormone, 7  
 — — clinical application of, 10  
 — — commercial preparations of, 356  
 — — effect on adrenal cortex, 85  
 — — relation to growth hormones, 9  
 — — — — thymus, 9  
 — — standardization of, 10  
 Adrenogenital syndrome, aetiology of, 79  
 — — endocrine system in, 217  
 Adrenolutein, 99, 174  
 Adrenosterone, 180  
 Adrenotrophic factor, 356  
 Adrenoxin, 91  
 Alarm reaction, 73, 82  
 Alloxan, 125  
 Alpha-oestradiol, administration of, in ointment, 156  
 — benzoate, administration of, intramuscular, 155  
 — dipropionate, administration of, intramuscular, 155  
 — suppositories, 155  
 Alpha-Tocopherol, and endocrine glands, 243  
 Ambinon "A" and "B", 356  
 Amenorrhoea due to increased oestrogen activity, treatment with progestogens, 176  
 — — — psychic trauma, 220  
 — hypo-oestrogenic, treatment with oestrogens, 157  
 — secondary, due to ovarian deficiency, treatment with progestogens, 176  
 — treatment of, with thyroid hormone, 53  
 Aminothiazol, 230  
 Amniotin, 361  
 Anaemia, macrocytic, treatment with androgens, 193  
 Andrenosterone, 70  
 Androgens, 180-203  
 — action of, mechanism of, 185  
 — assay methods, 275  
 — clinical applications and dosage, 191  
 — — — in women, 195  
 — commercial preparations of, 367  
 — effects of vitamin deficiency on, 189  
 — — on vaginal epithelium, 294  
 — excretion, normal values for, 276  
 — in treatment of pituitary dwarfism, 4  
 — inhibitory effect on spermatogenesis, 182  
 — oestrogenic effect, 184  
 — preparations of, for clinical use, 190  
 — relationship to endocrine glands, 187  
 — — — growth hormone, 3  
 — — — progestogen, 174  
 — secretion rate, 181  
 — standardization of, 190  
 Androsterone, 180  
 — commercial preparations of, 367  
 — standardization of, 190  
 Aneurin, see Vitamin B<sub>1</sub>  
 Angina pectoris, treatment of, with thiouracil, 225  
 Angina-like pain in male climacteric, treatment with testosterone propionate, 193  
 Angioneurotic oedema, treatment of, with adrenalin, 109

Anhydrohydroxy-progesterone, see Ethisterone  
 Anorexia nervosa, treatment with corticotrophic hormone, 10  
 Anoxia, effect on adrenal cortex, 72  
 Anteron, 357  
 Antex, 357  
 Anti-hormones, 26  
 Antostab, 356  
 Antroidin, 357  
 Antuitrin "S", 355, 357  
 A.P.L., 357  
 Arteriosclerosis obliterans, treatment of, with desoxycorticosterone acetate, 107  
 Aschheim-Zondek test for blood gonadotrophin, Fluhmann's modification, 248  
 — test for pregnancy, modification of, 257  
 Ascorbic acid, see Vitamin C.  
 Asthma, bronchial, treatment with adrenalin, 108  
 — — with impotence, treatment with testosterone propionate, 194  
 — treatment of, with adrenal hormone, 108  
 A.T.10, see Dihydrotachysterol  
 Atmospheric pressure, effect on endocrine function, 210  
 Azoospermia, effect of equine gonadotrophic hormone on, 23

## B

Basal metabolism, relation to adrenal cortex, 77  
 Basophil cells, 1  
 Ben-ovocyclin, 362  
 Benzoate unit, International, 147  
 Benzoestrol, 154, 366  
 Benzo-Gynoestryl, 362  
 Best's carmine method, modified, for staining endocervical smears, 314  
 "Bleeding Factor" in menstruation, 135  
 Blood calcium, effect of oestrogen on, 145  
 — gonadotrophin assay in non-pregnant conditions, 248

Blood, oestrogen assay in, 265  
 — sugar level, effect of oestrogen on, 123, 145  
 — — — maintenance of, rôle of insulin in, 119  
 — vessels, effects of adrenalin on, 91  
 — — — — oestrogens on, 138  
 Body measurements in relation to age, 346  
 — temperature during menstrual cycle, 316  
 Bone, metaplasia of, effect of oestrogen on, 145  
 Breast, cancer of, treatment with testosterone propionate, 99  
 — carcinoma of, treatment with oestrogens, 162  
 — effect of oestrogens on, 137  
 — — — progestin on, 173  
 — engorgement, post-partum, treatment with antrogens, 199  
 — — treatment and prophylaxis with oestrogens, 160  
 — mastopathia, premenstrual, treatment with testosterone propionate, 197  
 Burns, treatment of, with cortical extract, 107

## C

Cachexia, hypophyseal, see Simmonds's disease  
 Calciferol, see Vitamin D<sub>2</sub>  
 Calcium excretion, increase of, in hyperparathyroidism, 58  
 — metabolism, effect of oestrogens on, 138  
 — — — — thyroid hormone on, 45  
 Cannon's emergency reaction, 213  
 Capon comb test for estimation of androgens, 275  
 Carbohydrate metabolism, effect of adrenal cortical extract on, 76  
 — metabolism, effect of androgens on, 186  
 — — — — thyroid hormone on, 45  
 — — rôle of vitamin B in, 238  
 Cartland-Nelson rat unit, 20  
 Castration, effect on adrenal cortex, 85  
 — effects of, 186

- Cataract, in hypoparathyroidism, 59  
 Catarrh, vernal, treatment of, with adrenal hormone, 108  
 Cervical-seminal compatability tests, 331  
 Cervix uteri, see Uterus, cervix  
 Chloride plasma level, effect of desoxycorticosterone acetate on, 75  
 Chlorides, urinary, assay of, 288  
 Choline, 82  
 Chorionic gonadotrophic hormone, 17  
 — — — combination with pituitary gonadotrophic hormone, 20  
 — gonadotrophin, hormone, preparations of, for clinical use, 21  
 — — — standardization of, 19  
 — — hyperaemia test for, 255  
 Chromatophore-expanding action of posterior lobe extracts, 41  
 Chromophil cells, 1  
 Chromophobe cells, 1  
 Circulatory failure, acute, treatment of, with adrenalin, 109  
 — system, effect of thyroid hormone on, 46  
 Clauberg, progesterone unit, 175  
 Climacteric, effects of, on vaginal mucosa, 301  
 — hormonal balance during, 216  
 — male, treatment of, with testosterone propionate, 192, 193  
 Clinestrol, 364, 365  
 Cold test for assay of adrenal cortical steroids, 286  
 Cole-Saunders rat unit, 19  
 Colorimetric estimation of 17-ketosteroids, 281  
 Compound A, cortical extract, 70  
 — B, cortical extract, 70  
 — E, cortical extract, 71  
 — F, cortical extract, 71  
 Constipation, in hypoparathyroidism, 59  
 Corner and Allen progesterone unit, 175  
 Corpus luteum, formation by oestrogen administration, 142  
 Cortate, 359  
 Corticoids, adrenal, estimation of, 284  
 Corticosterone, 70  
 Corticotrophic hormone, 10; see Adrenocorticotrophic hormone  
 Cortin, 359  
 Cretinism, treatment of, with thyroid hormone, 53  
 Cryptorchidism, treatment with gonadotrophins, 31  
 Cushing's syndrome, 72; see also Pituitary basophilism  
 — — aetiology of, 78  
 — — oestrogen excess in, 145  
 — — treatment with androgens, 194  
 Cutler-Power-Wilder test for urinary chlorides, 288
- ### D
- D.B.E., administration of, 154  
 Dehydroandrosterone, 180  
 Dementia praecox, effect on endocrine function, 220  
 Desoxycorticosterone, 70  
 — acetate, 86  
 — — absorption rate of, 104  
 — — action of, 75  
 — — administration, effects of, 86  
 — — clinical application of, 105  
 — — effect on posterior pituitary, 39  
 — — overdosage, effects of, 87  
 — — pellet implantation of, 104  
 — — preparations of, for clinical use, 103  
 — — prevention of shock by, 81  
 — — standardization of, 101  
 Dextrose tolerance test, 120  
 Diabetes, control by vitamin B, 238  
 — effect of oestrogen in, 145  
 — — — thiouracil in, 225  
 — experimental, and adrenal cortex, 76  
 — mellitus, treatment with insulin 129  
 — — — — posterior pituitary lobe extracts, 43  
 Diagnostic procedures in endocrinology, 248-345  
 Dienoestrol, administration of, 154  
 — tablets, 366



- Diethyl thiourea, 229  
 Diethylstilboestrol, absorption rate, 137  
 — administration of, in ointment, 156  
 — — in suppositories, 156  
 — commercial preparations of, 364, 365  
 — effect on spermatogenesis, 137  
 — inactivation of, 138  
 Dihydrocorticosterone, 70  
 Dihydrotachysterol, 358  
 — dosage of, 63  
 — preparations of, for clinical use, 63  
 Di-insulin, preparation of, for clinical use, 128  
 Diiodotyrosine, 44  
 Di-menformon, 362, 363  
 Di-ovocyclin, 363  
 Doca, 359  
 Drug addiction, treatment of, with insulin, 130  
 Duodenal ulcer, treatment of, with desoxycorticosterone acetate, 107  
 Dwarfism, pituitary, 4  
 Dysmenorrhoea, effect of progestogens in, 176  
 — treatment with insulin, 130  
 — — with oestrogens, 159  
 — — — testosterone propionate, 197  
 Dyspareunia, treatment of, with androgens, 198

## E

- Ecto-gynoestryl, 363  
 Ecto-sterandryl, 367  
 Eczema, treatment of, with adrenal hormone, 108  
 Electroencephalogram in endocrine disturbances, 219  
 Electrolyte metabolism, effect of androgens on, 187  
 — — — oestrogens on, 138  
 — — rôle of adrenal cortex in, 74  
 Electrometric method for determination of ovulation time, 329  
 Emmenoplex, 364  
 Emotional upsets, 212

- Endocervical smears, 304  
 Endocrine disorders, complexity of, 28  
 — — treatment of, with non-hormonal substances, 222-234  
 — extracts, commercial preparations of, 355-368  
 — glands, environmental influences on, 210  
 — — psycho-somatic relationships, 212-221  
 — system, in mental disorders, 217  
 — relationship to vitamins, 235-247  
 Endometrial biopsy for determination of ovulation time, 323  
 Endometriosis, treatment of, with testosterone propionate, 198  
 Endometrium, biopsy of, 292  
 — effect of oestrogen on, 134  
 — — — progestogens on, 172  
 Enuresis, treatment with androgens, 194  
 Environment, effect on endocrine function, 210  
 Eosinophil cells, 1  
 Epididymis, puncture, technique of, 335  
 Epilepsy, endocrine system in, 217  
 Epinephrine, see Adrenalin  
 Epiphyses, age at closure of, 348  
 Equine gonadotrophic hormone, 18  
 — — — preparations of, for clinical use, 22  
 — — — standardization, 19  
 Ergosterol, irradiated, commercial preparations of, 358  
 Erugon, 367  
 Eschatin, 359  
 Estrone, 361  
 Ethinyl oestradiol, 363  
 — — administration of, 152  
 — testosterone, see Ethisterone  
 Ethisterone, 169  
 — preparations of, for clinical use, 175  
 — tablets, 367  
 Eucortone, 359  
 Euglobulin, formation of, 135  
 Eunuchoidism, 187  
 — treatment with gonadotrophins, 31  
 — — with testosterone, 191

Exophthalmos, effect of thiouracil on, 225

## F

Fallopian tubes, carcinoma of, treatment with testosterone propionate, 199

— — effect of oestrogen on, 136

— — — — progestin on, 173

— — insufflation, technique of, 330

— — non-patency of, treatment with oestrogens, 159

Fat Metabolism, effect of adrenal cortex on, 77

— — — — thyroid hormone on, 45

Féminization in the male, 79

Fibrillation, auricular, effect of thiouracil on, 225

Fluhmann's modified Aschheim-Zondek test, 248

Fluorimetric assay of oestrogens, 268

Folic acid, effect on oestrogenic activity, 140, 241

Follicle-stimulating hormone, 2, 13

— — assay in urine, 250

— — during follicular phase of menstrual cycle, 136

— — effect on oestrogen secretion, 133

— — effect on testicular function, 181

Föllutein, 357

Fractures, treatment with testosterone propionate, 195

Freemartin twin, relationship to androgenic secretion, 186

Friedman test for pregnancy, 258

Frigidity, sexual, treatment with oestrogens, 159

Fröhlich's syndrome, 28

Fuchsinophil reaction, in adrenal virilism, 79

— substance in adrenal cortex, 69

Furunculosis, treatment of, with thyroid hormone, 53

## G

Gastric ulcer, treatment of, with desoxycorticosterone acetate, 107

Gastro-intestinal tract, effect of posterior pituitary hormones on, 40

Geriatric fatiguability, treatment with methyl testosterone, 192

Gestyl, 356

Gierke's disease, 76

Gingivitis, senile and atrophic, treatment with oestrogens, 161

Glaucoma, treatment of, with adrenal hormone, 108

Globin, zinc insulin, 360

Gluconeogenesis, 122

— production by adrenal cortex, mechanism of, 76

Glutathione, 82

Glycogen, deposition in vagina, 137

— vaginal, variations in, 298

Glycosuria, effect of vitamin B on, 238

Goitre, effect of thiouracil on, 225

Gonadal system, effects of diabetes on, 123

— — — — oestrogen on, 144

— — — — pineal extract on, 204

— — relation of adrenal cortex to, 78

— — — — of adrenal gland to, 99

— — — — parathyroid to, 60

— — — — thymus to, 206

Gonadogen, 357

Gonadotrophic assay in urine, 249

— — — — as test for pregnancy, 257

— hormone from pregnant mares' serum, commercial preparations of, 356

— — preparations of, for clinical use, 20

— — standardization of, 19

— — synergic phenomena, 18

— hormones, 13

— — application of, in female, 26

— — — — in male, 31

— — assay methods for, 17, 248-57

Gonadotrophin, chorionic, hyperaemia test for, 255

— excretion, menopausal values for, 257

— — normal values for, 256

— — pregnancy values for, 256

Gonadotrophin excretion rate, determination of ovulation time by, 328  
 — hormone from pregnancy urine, commercial preparations of, 357  
 — see Gonadotrophic hormones  
 — tests for, 248-57  
 Gonadotrophins, assay, South African male frog test for, 254  
 Gonadyl, 356  
 Gonan, 357  
 Growth, effect of adrenal cortex on, 78  
 — — — androgens on, 187  
 — — — lactogenic hormone on, 11  
 — — — thymus on, 205  
 — hormone, 2  
 — — commercial preparations of, 355  
 — — dosage, 4  
 — — effect of oestrogen on, 144  
 — — — on insulin secretion, 125  
 — — preparations of, for clinical use, 3  
 — — relation to adrenocorticotrophic hormone, 9  
 — — relation to other hormones, 3  
 — — standardization, 3  
 Gynoestrol, 363  
 Gynoestryl, 363

## H

Haemopoiesis, effect of androgens on, 187  
 Haemopoietic system, relation to adrenal cortex, 77  
 Haemorrhage, use of adrenalin in, 108  
 Haemospermia, chronic, treatment with oestrogens, 163  
 Hair, in hypoparathyroidism, 59  
 Hamblen method for staining spermatozoa, 333  
 Hay fever, treatment of, with adrenal hormone, 108  
 Heart block, treatment of, with adrenalin, 109  
 — failure, congestive, treatment with thiouracil, 225  
 — — treatment of, with adrenalin, 109

Height, normal, in relation to age, 346  
 Hexoestrol, 366  
 — administration of, 154  
 Histidine pregnancy test, 265  
 Homosexuality, effect of androgen administration on, 192  
 Hormonal changes, effects of, on the psyche, 213  
 Hormones, antagonism to vitamins, 235  
 — commercial preparations of, 355-368  
 — relationship to vitamins, 235-247  
 — see also under the names of the various hormones  
 Huhner test for seminal compatibility, 331  
 Hyaluronidase, determination in semen and tissue extracts by turbidimetry, 336  
 — — rat-ova test, 337  
 Hydroxycorticosterone, 71  
 17-Hydroxy-11-Dehydrocorticosterone, 71  
 11-Hydroxyisoandrosterone, 71  
 17-Hydroxyprogesterone, 71  
 Hyperaemia test for chorionic gonadotrophin, 255  
 Hyperemesis gravidarum, treatment with adrenal hormones, 106  
 Hyperparathyroidism, 58  
 — effect on gonadal system, 60  
 — primary, 57  
 — — treatment of, 64  
 Hyperpituitarism, association with hyperglycaemia, 121  
 Hyperpyrexia, diabetic, 122  
 Hypertension, treatment of, with testosterone propionate, 193  
 Hyperthyroidism, effect of thiouracil in, 225  
 — — on nervous system, 218  
 — thymus in, 206  
 — treatment of, with oestrogens, 162  
 Hypocalcaemia, effects of, 59  
 Hypoglycaemia, effects on nervous system, 218  
 — treatment of, with adrenalin, 109  
 Hypogonadism, treatment of, with androgens, 191



- Hypomenorrhoea, treatment of, with  
oestrogens, 158  
— — — — thyroid hormone, 53  
Hypoparathyroidism, 59  
— treatment of, 65  
Hypophyseal cachexia, see Sim-  
monds's disease  
Hypophysis, see Pituitary  
Hypothyroidism, and adrenal corti-  
cal atrophy, 73  
— effects on sex glands, 49  
Hypothyroidism, 7  
Hysterosalpingography, 330

## I

- Iletin, 360  
Immune reactions, effect of alarm  
reaction on, 84  
Infant, premature, treatment of, with  
methyl testosterone, 195  
Infantilism, sexual, treatment of,  
with oestrogens, 157  
Inhibin, 181  
Insomnia, in hypoparathyroidism,  
59  
Insufflation, perirenal, 290  
Insulin, administration, 129  
— clinical application and dosage,  
129  
— commercial preparations of, 360  
— crystalline, soluble preparation of,  
for clinical use, 127  
— di-insulin, preparation of, for  
clinical use, 128  
— effect on adrenal cortex, 9, 85  
— functions, 119  
— globin zinc, preparation of, for  
clinical use, 128  
— preparations of, for clinical use,  
126, 360  
— protamine zinc, preparation of,  
for clinical use, 127  
— regular, preparation of, for clin-  
ical use, 126  
— secretion, control of, 118  
— standardization of, 126  
— unmodified, preparation of, for  
clinical use, 126  
Intermedin, 41  
International benzoate unit, 147  
— unit of progesterone, 175

- Interstitial-cell-stimulating hormone  
of testis, 181  
Iodine, effect on thyroid gland, 223  
Isoandrosterone, 70

## K

- Kapeller-Adler test for pregnancy,  
265  
Kendall's compound E, 71  
17-Ketosteroids, assay methods, 276  
— excretion, normal values for, 73,  
287  
Kidney function, effect of thyroid  
hormone on, 46  
Knaus's test for determination of  
ovulation time, 329  
Kolpon vaginal bougies, 361  
— — tablets and inserts, 362  
Kraurosis valvae, treatment of, with  
oestrogens, 162

## L

- Labour, induction of, use of oestro-  
gens in, 160  
Lactation, deficient, treatment with  
lactogenic hormone, 12  
— effect of vitamin E administration  
on, 245  
— inhibition of, by oestrogen, 143  
— — — use of oestrogens for, 160  
— rôle of adrenal cortex in, 80, 100  
Lactogenic hormone, 10  
Lactogenic hormone administration,  
12  
— — clinical applications, 12  
— — commercial preparations of,  
356  
— — effect of oestrogen on, 143  
— — relation to other glands, 11  
— — standardization of, 12  
Langerhans, islets of, 118, 189  
Leydig cells, androgen production  
by, 180  
Libido, decreased, in female, treat-  
ment with androgens, 198  
Light, effect on hypophyseal activity,  
210  
Lipemia in diabetic coma, 122  
Lipocaic, 129

Lipo lutin 366  
 Liver, function, effect of vitamin B deficiency on, 139  
 — inactivation of oestrogens by, 138  
 — relation to pancreas, 120  
 Luteinization, relation to oestrogenic activity, 134  
 Luteinizing hormone, 2, 13  
 — — during follicular phase of menstrual cycle, 136  
 — — effect on oestrogen secretion, 133  
 — — — — testicular function, 181  
 Luteocyclin, 366, 367  
 Luteostab, 366  
 Luteotrophic principle, 134  
 Luteotropin, 10, 136, 356  
 Lutocyclol, 367  
 Lutogyl, 367  
 Lymphocytes, adrenocorticotrophic hormone control of, 7  
 Lynoral, 362

## M

Mack's test for cellular glycogen in endocervical smears, 315  
 Male, feminization in, 79  
 Malnutrition, treatment of, with insulin, 130  
 Marcrogenitosomia praecox, 204  
 Maternal feeling, relationship to menstrual flow, 216  
 Melancholia, involutional, treatment with oestrogens, 161  
 Melancholic depression, treatment with corticotrophic hormone, 10  
 Melanophore-expanding principle of pars intermedia, 41  
 Menformon, 361, 362  
 Menometrorrhagia, 27  
 Menopausal flushes, treatment with vitamin E, 245  
 — symptoms, alleviation of, with oestrogens, 160  
 Menopause, see Climacteric, 216  
 Menorrhagia, treatment of, with androgens, 197  
 — — — — oestrogens, 158  
 — — with lactogenic hormone, 13

Menstrual cycle, changes of vaginal mucosa in, 295  
 — — emotional changes during, 213  
 — — follicular phase, oestradiol secretion in, 135  
 — — hormonal balance during, 213  
 — — non-ovulatory, oestrogen in, 136  
 — — temperature fluctuations during, 316  
 — — vitamin C level during, 24  
 — disorders, aetiology of, 28  
 — terminology of, 27  
 — treatment of, with androgens, 196  
 — — — — oestrogens, 158  
 — — — with gonadotrophin, 26  
 — — — thyroid hormone, 53  
 — disturbances, due to Vitamin B deficiency, 139  
 Menstruation, disorders of, effect of Vitamin E on, 245  
 — effect on electroencephalogram, 219  
 — mechanism of, 135  
 Mental disorders, endocrine system in, 217  
 — disturbances, relationship to endocrines, 212  
 Metabolism, basal, clinical significance of, 320  
 — — determination of, 319  
 — — effect of oestrogen on, 144  
 — — — — thiouracil on, 228  
 — — — — thyroid hormone on, 44  
 Metandren, 368  
 Methyl-testosterone, 180  
 — clinical use, 190  
 — commercial preparations of, 368  
 Methyl-thiouracil, 229  
 — commercial preparations of, 368  
 Metropathia haemorrhagica, treatment of, with oestrogens, 158  
 — — — — progestogens, 177  
 Metrorrhagia, treatment of, with oestrogens, 158  
 Miller-Kurzrok test for cervical-seminal compatibility, 331  
 Mucous membranes, congestion of, treatment with adrenalin, 108

- Mucus, cervical, normal values, 332
- Muscle, effects of adrenalin on, 91
- Muscles, in hypoparathyroidism, 59
- Muscular system, effect of thyroid hormone on, 46
- Myasthenia gravis, rôle of thymus in, 205
- Myopia, progressive, treatment of, with adrenal hormone, 108
- Myxoedema, effect of thyrotrophic hormone in, 6
- — — on electroencephalogram, 219
- — — nervous system, 218

## N

- "N" hormone of adrenal cortex, 72
- Nails, in hypoparathyroidism, 59
- Neo-Hombreol, 367, 368
- Neo-oestranol-1, 365
- Nervous system, effect of thyroid hormone on, 46
- Neutrophil cells, 1
- Nicotinamide, effects in adrenalectomized rats, 101
- Nicotine, effect on posterior pituitary, 39
- Nitritoid crisis, post-arsphenamine, treatment of, with adrenalin, 109
- Nitrogen hormone of adrenal cortex, 72
- metabolism, effect of oestrogens on, 138
- Nymphomania, treatment of, with testosterone propionate, 198

## O

- Obesity, treatment of, with thyroid hormone, 52
- Octofollin, 154, 366
- Oestradiol, 133, 136, 137
- administration by implantation, 157
- — of, 152
- benzoate, administration, by implantation, 157
- — — of, in ointment, 156
- commercial preparations of, 363
- dipropionate, administration, by implantation, 157

- Oestradiol dipropionate, commercial preparations of, 363
- during follicular phase of menstrual cycle, 135
- in propylene glycol, administration of, 155
- inactivation of, 138
- relationship to androgens, 181
- Oestriol, 133, 136
- administration of, 153
- inactivation of, 138
- Oestroform, 362, 363
- Oestrogens, 133-68
- absorption rate, 152
- activity of, during child-bearing age, 133
- — — during pregnancy, 134
- — — in female, 133
- — — in male, 137
- administration, 147
- — implantation method, 148, 156
- — in ointments, 156
- — — suppositories, 155
- — — intramuscular, 155
- — — oral, 152
- assay methods, 265
- clinical applications and dosage, 157
- commercial preparations of, 361
- effects of, 138
- — — vitamin B on, 239
- — — on adrenal cortex, 8, 73, 85, 99
- — — blood-sugar level, 123
- — — eosinophil cells, 14
- — — lactogenic hormone, 11, 143
- — — thymus involution, 206
- — — thyroid function, 49
- — — vaginal epithelium, 293
- effects on female reproductive organs, 134
- excretion, 133
- — during pregnancy, 134
- — normal values for, 2, 137
- — rate, determination of ovulation time by, 328
- inactivation, 138
- relation to androgens, 189
- — — other endocrine glands, 140
- — — parathyroid, 60



- Oestrogens, relation to progestin, 174  
 — standardization, 147  
 Oestroglandol, 361, 362  
 Oestrone, 133, 136, 361  
 — administration, by implantation, 156  
 — — in ointment, 156  
 — — of intramuscular, 155  
 — — — oral, 152  
 — commercial preparations of, 363  
 — inactivation of, 138  
 Old age, fatiguability in, treatment with methyl testosterone, 192  
 Oligomenorrhoea, treatment of, with oestrogens, 158  
 — — with gonadotrophin, 30  
 — — — thyroid hormone, 53  
 Oligospermia, effect of equine gonadotrophic hormone on, 23  
 Oreton, 367, 368  
 Osseous centres, age at appearance of, 348  
 Ostelin, 359  
 Osteomalacia, treatment of, 65  
 Osteitis fibrosa cystica, 58, 145  
 Otosclerosis, treatment with testosterone propionate, 194  
 Ovariectomy, effect on pituitary gland, 140  
 Ovaries, cysts of, treatment with oestrogens, 159  
 — effect of oestrogen on, 146  
 — — — progestogen on, 174  
 Ovary, carcinoma of, treatment with testosterone propionate, 199  
 — hypofunction of, treatment with gonadotrophin, 30  
 — relationship to vitamin C, 242  
 — responsiveness to gonadotrophic therapy, test for, 292  
 Ovocyclin, 363, 364  
 Ovostab, 361, 362  
 Ovulation, haemorrhagic, painful, treatment with testosterone propionate, 198  
 — relation to oestrogenic activity, 134  
 — test, rapid, for diagnosis of pregnancy, 258  
 — time of, determination, 322  
 Oxytocic factor of pituitary, inhibition by progestin, 172, 173  
 — — — posterior pituitary, inhibition by testosterone propionate, 187
- ### P
- Pabestrol, 365  
 Pabestrosalve, 365  
 Pancreas, effect of oestrogens on, 145  
 — — — parathyroid hormone on, 60  
 — islet system of, 118-132  
 — — — — effect of androgens on, 189  
 — — system, physiology of, 119  
 — relationship to adrenal gland, 98  
 — — — thymus, 207  
 — — — thyroid gland, 48  
 Pantothenic acid deficiency, effects of, 100, 241  
 Papanicolaou's stain for endocervical smear, 310  
 — and Shorr's method for staining endocervical smears, 313  
 Paralysis, periodic, treatment with thyroid hormone, 53  
 Para-thor-mone, 359  
 Parathyroid gland, effects of cold on, 210  
 — — — — oestrogen on, 145  
 — — hormone commercial preparations of, 359  
 — — relationship to adrenals, 97  
 — — — — other glands, 59  
 — — — — vitamin D, 243  
 — hormone, 56-67  
 — — preparations for clinical use, 62  
 — — relationship to vitamin D, 60  
 — — standardization of, 61  
 — insufficiency, effect on electroencephalogram, 219  
 — relation to androgens, 188  
 — — — thyroid gland, 47  
 Paraxanthine, 229

- Pars intermedia of pituitary, hormones of, 41
- Pelvis, ligaments of, effect of progestin on, 173
- Peptic ulcer, treatment with posterior pituitary preparations, 43
- Perandren, 367
- Percorten, 359
- Perirenal insufflation, 290
- Pertussis, treatment of, with cortical extract, 107
- Pheochromocytoma, adrenal, effects of, 91
- Phosphatase level in serum, increase of, in hyperparathyroidism, 57
- Phosphate excretion, increase of, in hyperparathyroidism, 58
- Phosphorus level in serum, 56
- Phykentrone, 355
- Physolactin, 356
- Physostab, 357
- Pineal gland, 204
- — — extract, effect on gonadal function, 204
- Pitocin, 40, 357
- clinical application in obstetrical conditions, 42
- preparation of, for clinical use, 42
- standardization of, 41
- Pitone snuff, 358
- Pitressin, 38, 357
- effect on gastro-intestinal tract, 40
- in treatment of diabetes mellitus, 43
- — — post-operative abdominal complications, 43
- — — — pyelitis, 43
- preparations of, for clinical use, 42
- standardization of, 41
- Pituitary anterior lobe, effect of thiouracil on, 222
- — — effects on oestrogen secretion, 140
- — — effects of thyroxin on, 47
- — — extract, commercial preparations of, 355
- — — hormones of, 1-37
- — — relation to adrenals, 94
- — — — androgen secretion, 187
- Pituitary anterior lobe, relation to pancreas, 121
- — — — parathyroid, 59
- basophilism, 79; see also Cushing's syndrome, 79
- cachexia, see Simmonds's disease
- dwarfism, 4
- effects of thyroidectomy on, 46
- gland, effect of light on, 210
- — posterior, effect of oestrogen on, 144
- — — hormone, commercial preparations of, 357
- — — lobe, oxytocic factor of, inhibition by testosterone propionate, 187
- — — — relationship to adrenals, 39, 96
- — — oxytocic factor of, inhibition by progestin, 172, 173
- — rôle in defence reactions, 83
- gonadotrophic hormone, preparations of, for clinical use, 20
- — — standardization of, 19
- — hormones, 13, 355
- — — combination with chorionic gonadotrophic hormone, 20
- pars intermedia, hormones of, 41
- posterior, desiccated powder, 42, 43, 358
- — effect of pregnancy hormones on, 39
- — lobe, hormones of, 38-43
- Pituitrin, 38, 358
- clinical application in obstetrical conditions, 42
- effect on insulin, 122
- — on uterus, 40
- preparations of, for clinical use, 42
- standardization of, 41
- uterine reaction to, use as indication of ovulation time, 329
- Placental tissue, progestin in, 172
- Polyansyn, 355
- Polymenorrhoea, treatment with chorionic gonadotrophin, 30

- Post-coital test, Huhner's, 331
- Potassium plasma level, increase in  
adrenal cortical deficiency, 75
- Pranone, 367
- Pranturon, 357
- Pregnancy, adrenal cortical function  
in, 74
- diagnostic tests for, 257-265
- effect of androgens on, 186
- — — desoxycorticosterone on,  
86
- — on electroencephalogram, 219
- — — vaginal mucosa, 298
- oestrogenic activity in, 134
- progesterone secretion in, 169
- test, six-hour, 259
- — twenty-four hour, 260
- — two-hour, 259
- three-hour colour test for, 262
- thyroid tolerance in, 49
- toxæmias of, treatment with  
vitamin E, 244
- Pregnanediol, 99
- assay methods, 272
- excretion of, in pregnancy, 169
- — rate of, in determination of  
ovulation time, 326
- metabolism, 171
- normal values for, 275
- Pregnanetriol, 70
- Pregneninolone, see Ethisterone
- Pregnyl, 357
- Preloban, 355
- Premarin, 361
- Prematurity, treatment of, with  
methyl testosterone, 195
- Premenstrual tension, treatment of,  
with androgens, 197
- Progesterone, 70, 169
- action of, 135
- commercial preparations of, 366
- preparations of, for clinical use,  
175
- Progestin, 169, 366
- effect on eosinophil cells, 15
- effects on female reproductive  
organs, 172
- inhibition of posterior pituitary  
factors by, 135
- relationship to endocrine glands,  
173
- Progestogens, 169-179
- action of, effect of oestrogen on,  
146
- clinical application and dosage,  
176
- commercial preparations of, 266
- effects on female reproductive  
organs, 172
- effects on vaginal epithelium, 293
- metabolism of, 171
- physiology of, 169
- preparations of, for clinical use,  
175
- production of cortical atrophy by,  
73
- standardization of, 175
- Progestoral, 367
- Progynon, 361-4
- Prolactin, 10, 356
- Prolan, 357
- Proluton, 366, 367
- Propylthiouracil, 230, 368
- Prostate, carcinoma of, treatment  
with oestrogens, 163
- hypertrophy of, treatment with  
oestrogens, 163
- Prostigmine pregnancy test, 264
- Protamine zinc insulin, 127
- Protein metabolism, effect of adrenal  
cortical extract on, 76
- — effect of thyroid hormone on,  
45
- Proviron, 367
- Pruritus, senile, in the male, treat-  
ment with testosterone pro-  
pionate, 192
- vulvae, treatment of, with oestro-  
gens, 162
- Pseudocyesis, effect on endocrine  
function, 220
- Pseudo-hermaphroditism, treatment  
with chorionic gonadotrophin,  
31
- Psychoasthenia, in hypoparathyroid-  
ism, 59
- Psychoses, puerperal, 216
- relationship to endocrines, 212
- Psycho-somatic endocrinology, 212-  
221
- Puberty, delayed, treatment of, with  
oestrogens, 157



Puberty, delayed, treatment with testosterone propionate, 191  
 — psychological disturbances in, 213  
 Puerperal psychoses, 216  
 Pyelitis, treatment with pitressin, 43  
 Pyridoxin, effects on adrenals, 101  
 Pyruvic acid level in thyrotoxicosis, 51

## R

von Recklinghausen's disease, see Hyperparathyroidism  
 "Release phenomenon" of oestrogen, 142  
 Reproductive organs, female, effects of oestrogens on, 134  
 Resuscitation, use of adrenalin in, 108  
 Riboflavin, see Vitamin B<sub>2</sub>  
 Rickets, treatment of, 64  
 Robbie and Gibson's method for assay of 17-ketosteroids, 278  
 Roentgen sickness, treatment of, with desoxycorticosterone acetate, 107

## S

"S" Hormone, 72  
 Schizophrenia, treatment of, with cortical extract, 107  
 — — — — insulin, 130  
 Scott's urine concentration test for pregnancy, 261  
 Sedo-gynoestryl, 364  
 Semen, examination of, 332  
 — normal values, 335  
 — post-coital examination of, 331  
 — see also Spermatozoa  
 Serogan, 357  
 Serum phosphatase level, increase of, in hyperparathyroidism, 57  
 — sickness, treatment of, with adrenalin, 109  
 Sex glands, relationship to thyroid gland, 48  
 Sexual infantilism, treatment of, with oestrogens, 157  
 Shock, prevention by decortico-sterone acetate, 81

Shorr's stain for endocervical smears, 313  
 Simmonds's disease, 28  
 — — and adrenal cortex, 73  
 — — treatment with methyl testosterone, 195  
 Skin, in hypoparathyroidism, 59  
 Sodium oestrone sulphate, administration of, 153  
 — plasma level, effect of desoxycorticosterone, acetate on, 75  
 — pregnanediol glycuronide, 171  
 South African male frog test for gonadotrophins, 254  
 Spermatogenesis, 180  
 — deficient, treatment with equine gonadotrophic hormone, 23, 31  
 — effect of diethylstilboestrol on, 137  
 — inhibitory effect of androgens on, 182  
 Spermatozoa, microscopic appearance of, 334  
 — motility, estimation of, 332  
 — normal count, 335  
 — staining methods, 333  
 — stimulating action of androgens on, 183  
 — viability, determination of, 333  
 Status asthmaticus, treatment with adrenalin, 108  
 — thymicolymphaticus, 206  
 Staub-Traugott effect, 121  
 Sterandryl, 367  
 Sterility, female, treatment with thyroid hormone, 53  
 — male, effect of vitamin B on, 240  
 — primary, relationship to vitamin E, 246  
 Stilboestrol, administration of, 153  
 — commercial preparations of, 364, 365  
 — in treatment of prostatic carcinoma, 163  
 — inactivation by liver, 239  
 Sudanophobic unit, 10  
 Sugar hormone, 72  
 — tolerance in thyroid disease, 48  
 Sulkowitch's reagent and test, 64  
 Suprarenal gland, see Adrenal gland  
 Sympathin, 90

Synapoidon, 356

— preparations of, for clinical use, 24

— standardization of, 20

Synthovo, 366

## T

Temperature, body, basal, graphs of, for determination of ovulation time, 327

— — during menstrual cycle, 316

— effect on endocrine function, 210

Testicle, biopsy, technique of, 335

— puncture, technique of, 335

Testis, effect of oestrogens on, 146

— function of, control of, 180

— germinal epithelium of, production of oestrogens by, 137

— interstitial-cell-stimulating hormone, 181

Testosterone, 180

— clinical applications, 191

— implantation, preparations for, 190

— in aqueous suspension, preparations of, for clinical use, 190

— — propylene glycol-alcohol, preparations of, for clinical use, 190

— oestrogenic effect, 184

— production of cortical atrophy by, 73

— propionate, 180

— — clinical application, 190, 191

— — commercial preparations of, 367

— — implantation, preparations for, 190

— — inhibitory effect on spermatogenesis, 182

Testoviron, 367

Tetany, parathyroid, 59

Tetramethyl thiourea, 229

Theelin, 361, 362

Theelol, 364

Thiamin, see Vitamin B<sub>1</sub>

Thiobarbitone, 230

Thiouracil, 222

— commercial preparations of, 368

— dosage, 227

— effect on anterior pituitary, 222

Thiouracil, effect on exophthalmos and goitre, 225

— effects on adrenal cortex, 95

— in pregnancy, 224

— mode of action, 222

— pre-operative use, 228

— therapeutic effects, 224

— toxic effects, 226

Thiourea, 229

Thrombo-angiitis obliterans, treatment of, with desoxycorticosterone acetate, 107

Thyroxinsodium, standardization of, 51

Thymotrophic hormone, 206

Thymus, 205

— changes in, during alarm reaction, 83

— effect of androgens on, 188

— gland, relation to other endocrine glands, 206

— involution after hypophysectomy, 206

— relation to adrenocorticotrophic hormone, 9

— relationship to adrenal gland, 98

— — — thyroid gland, 49

Thyrogan, 355

Thyroglobulin, 44

Thyroid gland, desiccated, preparations of, for clinical use, 52

— — — standardization of, 51

— — — tablets, 358

— — — effect of androgens on, 188

— — — — oestrogen on, 144

— — — — progestin on, 173

— — — — temperature on, 210

— — — function, evaluation of, by aspiration biopsy, 321

— — — hormone, commercial preparations of, 358

— — — relationship to growth hormone, 3

— — — tolerance to, in pregnancy, 49

— — — hyperplasia of, due to thiouracil, 227

— — — in mental disorders, 218

— — — physiology of, 44

- Thyroid gland, relation to adrenals, 96  
 — — — — other endocrine glands, 46  
 — — — — parathyroid, 59  
 — — — — thymus, 206  
 — — — — vitamins, 50, 236, 237, 240  
 — hormone, 44-55  
 — — clinical applications, 52  
 — — effect on adrenal cortex, 85  
 — — — — lactogenic hormone, 12  
 — — — — pancreas, 123  
 — — preparations for clinical use, 52  
 — — standardization of, 51  
 Thyroidectomy, effect on pituitary gland, 46, 140  
 Thyrotoxicosis, treatment with androgens, 195  
 — — — vitamin B<sub>1</sub>, 50  
 Thyrotrophic factor, 355  
 — hormone, 5  
 — — clinical application, 7  
 — — commercial preparations of, 355  
 — — dosage, 6  
 — — standardization of, 6  
 Thyrotropin, 355  
 Thyroxin, 44, 358  
 — effect on adrenal cortex, 9, 73  
 — preparations of, for clinical use, 52  
 — standardization of, 52  
 Tocopherols, and endocrine glands, 243  
 Tuberculosis, pulmonary, chronic, effect on adrenal cortex, 73

## U

- Unden, 361, 362  
 Urine, chlorides in, estimation of, 288  
 — follicle-stimulating hormone in, assay of, 250  
 — "free" oestrogen in, assay of, 268  
 — gonadotrophin assay, in non-pregnant conditions, 249  
 — — — in pregnancy, 257  
 — — excretion rate, use in determination of ovulation time, 328

- Urine, 17-ketosteroid estimation in, 276  
 — oestrogen assay in, 267  
 — — excretion rate use in determination of ovulation time, 328  
 Urticaria, treatment of, with adrenalin, 108  
 Uterine bleeding, functional, treatment with thyroid hormone, 53  
 — — non-ovulatory, treatment with gonadotrophic hormones, 30  
 Uterus, bleeding, functional, due to protracted oestrogenic action, treatment with progestogens, 177  
 — carcinoma of, treatment with testosterone propionate, 199  
 — cervix, acid mucosa of, treatment with progestogens, 178  
 — — carcinoma of, treatment with testosterone propionate, 199  
 — — — vaginal smear in, 301  
 — — endocervical smears, preparation of, 304  
 — — mucosa of, effect of progestin on, 173  
 — — mucus, normal values, 332  
 — effect of oestrogens on, 134  
 — — — pituitrin on, 40  
 — endometrial biopsy, for determination of ovulation time, 323  
 — endometrium, biopsy of, 292  
 — fibroids, treatment with testosterone propionate, 198  
 — fibromyoma, treatment of, with progesterone, 178  
 — functional bleeding of, treatment with lactogenic hormone, 13  
 — — — treatment of, with androgens, 195  
 — hysterosalpingography, 330  
 — mucosa, effect of progestogens on, 172

## V

- Vagina, acidity of, maintenance of, 137  
 — effects of oestrogen on, 137



Vagina, effects of progestin on, 173  
 — glycogen concentration, variations in, 298  
 — smear, 292  
 — — after androgen administration, 184  
 — — changes during menstrual cycle, 295  
 — — characteristics of, 295  
 — — in carcinoma uteri, 301  
 — — — menopause, 301  
 — — — pregnancy, 298  
 — — use in determination of ovulation time, 327  
 Vaginitis, senile, treatment with oestrogens, 161  
 Venning's pregnanediol assay method, 272, 273  
 Viosterol, preparations of, for clinical use, 63  
 Virilism, adrenal, 70, 73, 78, 79, 80  
 — oestrogen excess in, 145  
 Vitamin A, relation to thyroid gland, 50, 236  
 — — storage of, by adrenals, 100  
 — B, and carbohydrate metabolism, 238  
 — — deficiency, relation to adrenal cortex, 100  
 — — effect on oestrogens, 239  
 — — rôle in carbohydrate metabolism, 125  
 — — — — oestrogen inactivation, 139  
 — — complex, deficiency, effects on adrenals, 100  
 — B<sub>1</sub> in relationship to thyroid gland, 50, 237  
 — — — treatment of diabetes, 126  
 — — — — thyrotoxicosis, 50  
 — B<sub>2</sub> deficiency, effects on adrenals, 100  
 — — functions of, 240  
 — — phosphorylation, 240  
 — C, concentration, adrenal, effect of adrenocorticotrophic hormone on, 7  
 — — effects on adrenal hormones, 101  
 — — relationship to endocrines, 242  
 — — — — thyroid gland, 51

Vitamin D, clinical applications, 64  
 — — effect on thyroid gland, 51  
 — — effects of excessive amounts of, 61  
 — — preparations of, for clinical use, 62  
 — — relationship to parathyroid hormone, 60, 243  
 — — standardization of, 61  
 — D<sub>1</sub>, 62  
 — D<sub>2</sub>, preparations of, for clinical use, 62  
 — D<sub>3</sub>, preparations of, for clinical use, 63  
 — E, antagonism of, to oestrogens, 147  
 — — deficiency, testicular effects, 189  
 — — effect on acetylcholine synthesis, 207  
 — — relationship to endocrines, 243  
 Vitamins, and adrenals, 100  
 — antagonism to hormones, 235  
 — deficiency, androgenic effects, 189  
 — relationship to endocrines, 235—247  
 — — — thymus, 207  
 — — — thyroid gland, 50  
 Vomiting, recurrent, treatment of, with insulin, 130

## W

Warren's method for assay of 17-ketosteroids, 280  
 Water intoxication, 217  
 — metabolism, effect of androgens on, 187  
 — — — — oestrogens on, 138  
 — — — — thyroid hormone on, 45  
 — rôle of adrenal cortex in, 74  
 "Water test" for diagnosis of Addison's disease, 288  
 Waterhouse-Friederichsen syndrome, treatment of, with adrenal hormones, 106  
 Weight, normal, in relation to age, 346  
 Whooping cough, treatment of, with cortical extract, 107  
 Wooster's method for assay of 17-ketosteroids, 277

## X

"X" zone of adrenal cortex, 69

*Xenopus laevis*, gonadotrophin test,

254

— — pregnancy test, 260

## Y

Yohimbine, therapeutic uses of, 231

## Z

Zona reticularis of adrenal cortex,  
70





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